

CRISPR and Cancer Biology Research Literatures

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Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. Clustered regularly interspaced short palindromic repeats (CRISPR) are segments of prokaryotic DNA containing short, repetitive base sequences. Each repetition is followed by short segments of spacer DNA from previous exposures to foreign DNA. Small clusters of *cas* (CRISPR-associated system) genes are located next to CRISPR sequences. CRISPR-Cas9 is a new powerful technique for the gene editing target. This article introduces recent research reports as references in the related studies.

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Key words: cancer; life; research; literature; cell; CRISPR; Cas9

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. Clustered regularly interspaced short palindromic repeats (CRISPR) are segments of prokaryotic DNA containing short, repetitive base sequences. Each repetition is followed by short segments of spacer DNA from previous exposures to foreign DNA. Small clusters of *cas* (CRISPR-associated system) genes are located next to CRISPR sequences. CRISPR-Cas9 is a new powerful technique for the gene editing target.

The following introduces recent reports as references in the related studies.

Ahn, J., K. H. Kim, et al. "Target sequencing and CRISPR/Cas editing reveal simultaneous loss of UTX and UTY in urothelial bladder cancer." *Oncotarget*. 2016 Sep 27;7(39):63252-63260. doi: [10.18632/oncotarget.11207](https://doi.org/10.18632/oncotarget.11207).

UTX is a histone demethylase gene located on the X chromosome and is a frequently mutated

gene in urothelial bladder cancer (UBC). UTY is a paralog of UTX located on the Y chromosome. We performed target capture sequencing on 128 genes in 40 non-metastatic UBC patients. UTX was the most frequently mutated gene (30%, 12/40). Of the genetic alterations identified, 75% were truncating mutations. UTY copy number loss was detected in 8 male patients (22.8%, 8/35). Of the 9 male patients with UTX mutations, 6 also had copy number loss (66.7%). To evaluate the functional roles of UTX and UTY in tumor progression, we designed UTX and UTY single knockout and UTX-UTY double knockout experiments using a CRISPR/Cas9 lentiviral system, and compared the proliferative capacities of two UBC cell lines in vitro. Single UTX or UTY knockout increased cell proliferation as compared to UTX-UTY wild-type cells. UTX-UTY double knockout cells exhibited greater proliferation than single knockout cells. These findings suggest both UTX and UTY function as dose-dependent suppressors of UBC development. While UTX escapes X chromosome inactivation in females, UTY may function as a male homologue of UTX, which could compensate for dosage imbalances.

Akincilar, S. C., E. Khattar, et al. "Long-Range Chromatin Interactions Drive Mutant TERT Promoter Activation." *Cancer Discov*. 2016 Nov;6(11):1276-1291. Epub 2016 Sep 20.

Cancer-specific TERT promoter mutations (-

146C>T and -124C>T) have been linked to reactivation of the epigenetically silenced telomerase reverse transcriptase gene (TERT). Understanding how these single-nucleotide alterations drive TERT reactivation is a fundamental unanswered question and is key for making successful therapeutics. We show that unlike wild-type promoters, recruitment of the transcription factor GABPA specifically to mutant TERT promoters mediates long-range chromatin interaction and enrichment of active histone marks, and hence drives TERT transcription. CRISPR-mediated reversal of mutant TERT promoters, or deletion of its long-range interacting chromatin, abrogates GABPA binding and long-range interactions, leading to depletion of active histone marks, loss of POL2 recruitment, and suppression of TERT transcription. In contrast, de novo introduction of a TERT promoter mutation enables GABPA binding and upregulation of TERT via long-range interactions, acquisition of active histone marks, and subsequent POL2 recruitment. This study provides a unifying mechanistic insight into activation of mutant TERT promoters across various human cancers. SIGNIFICANCE: This study identifies a key mechanism by which cancer-specific mutant TERT promoters cause reactivation of TERT. Because the mechanism uncovered here is not utilized by promoters that drive TERT in normal cells, this mechanism could be exploited to make inhibitors which have the potential to block telomerase function and hence the progression of up to 90% of human cancers. *Cancer Discov*; 6(11); 1276-91. (c)2016 AACR. See related commentary by Min and Shay, p. 1212. This article is highlighted in the In This Issue feature, p. 1197.

Antonicka, H., K. Choquet, et al. "A pseudouridine synthase module is essential for mitochondrial protein synthesis and cell viability." *EMBO Rep*. 2017 Jan;18(1):28-38. doi: 10.15252/embr.201643391. Epub 2016 Dec 14.

Pseudouridylation is a common post-transcriptional modification in RNA, but its functional consequences at the cellular level remain largely unknown. Using a proximity-biotinylation assay, we identified a protein module in mitochondrial RNA granules, platforms for post-transcriptional RNA modification and ribosome assembly, containing several proteins of unknown function including three uncharacterized pseudouridine synthases, TRUB2, RPUSD3, and RPUSD4. TRUB2 and RPUSD4 were previously identified as core essential genes in CRISPR/Cas9 screens. Depletion of the individual enzymes produced specific mitochondrial protein synthesis and oxidative phosphorylation assembly defects without affecting mitochondrial mRNA levels.

Investigation of the molecular targets in mitochondrial RNA by pseudouridine-Seq showed that RPUSD4 plays a role in the pseudouridylation of a single residue in the 16S rRNA, a modification that is essential for its stability and assembly into the mitochondrial ribosome, while TRUB2/RPUSD3 were similarly involved in pseudouridylating specific residues in mitochondrial mRNAs. These results establish essential roles for epitranscriptomic modification of mitochondrial RNA in mitochondrial protein synthesis, oxidative phosphorylation, and cell survival.

Arbab, M. and R. I. Sherwood "Self-Cloning CRISPR." *Curr Protoc Stem Cell Biol*. 2016 Aug 17;38:5B.5.1-5B.5.16. doi: 10.1002/cpsc.14.

CRISPR/Cas9-gene editing has emerged as a revolutionary technology to easily modify specific genomic loci by designing complementary sgRNA sequences and introducing these into cells along with Cas9. Self-cloning CRISPR/Cas9 (scCRISPR) uses a self-cleaving palindromic sgRNA plasmid (sgPal) that recombines with short PCR-amplified site-specific sgRNA sequences within the target cell by homologous recombination to circumvent the process of sgRNA plasmid construction. Through this mechanism, scCRISPR enables gene editing within 2 hr once sgRNA oligos are available, with high efficiency equivalent to conventional sgRNA targeting: >90% gene knockout in both mouse and human embryonic stem cells and cancer cell lines. Furthermore, using PCR-based addition of short homology arms, we achieve efficient site-specific knock-in of transgenes such as GFP without traditional plasmid cloning or genome-integrated selection cassette (2% to 4% knock-in rate). The methods in this paper describe the most rapid and efficient means of CRISPR gene editing. (c) 2016 by John Wiley & Sons, Inc.

Attayek, P. J., J. P. Waugh, et al. "Automated microraft platform to identify and collect non-adherent cells successfully gene-edited with CRISPR-Cas9." *Biosens Bioelectron*. 2016 Dec 10;91:175-182. doi: 10.1016/j.bios.2016.12.019.

Microraft arrays have been used to screen and then isolate adherent and non-adherent cells with very high efficiency and excellent viability; however, manual screening and isolation limits the throughput and utility of the technology. In this work, novel hardware and software were developed to automate the microraft array platform. The developed analysis software identified micrarafts on the array with greater than 99% sensitivity and cells on the micrarafts with 100% sensitivity. The software enabled time-lapse imaging and the use of temporally varying

characteristics as sort criteria. The automated hardware released micrafts with 98% efficiency and collected released micrafts with 100% efficiency. The automated system was used to examine the temporal variation in EGFP expression in cells transfected with CRISPR-Cas9 components for gene editing. Of 11,499 micrafts possessing a single cell, 220 micrafts were identified as possessing temporally varying EGFP-expression. Candidate cells (n=172) were released and collected from the micraft array and screened for the targeted gene mutation. Two cell colonies were successfully gene edited demonstrating the desired mutation.

Barrangou, R. and J. A. Doudna "Applications of CRISPR technologies in research and beyond." Nat Biotechnol. 2016 Sep 8;34(9):933-941. doi: 10.1038/nbt.3659.

Programmable DNA cleavage using CRISPR-Cas9 enables efficient, site-specific genome engineering in single cells and whole organisms. In the research arena, versatile CRISPR-enabled genome editing has been used in various ways, such as controlling transcription, modifying epigenomes, conducting genome-wide screens and imaging chromosomes. CRISPR systems are already being used to alleviate genetic disorders in animals and are likely to be employed soon in the clinic to treat human diseases of the eye and blood. Two clinical trials using CRISPR-Cas9 for targeted cancer therapies have been approved in China and the United States. Beyond biomedical applications, these tools are now being used to expedite crop and livestock breeding, engineer new antimicrobials and control disease-carrying insects with gene drives.

Barrow, J. J., E. Balsa, et al. "Bromodomain Inhibitors Correct Bioenergetic Deficiency Caused by Mitochondrial Disease Complex I Mutations." Mol Cell. 2016 Oct 6;64(1):163-175. doi: 10.1016/j.molcel.2016.08.023. Epub 2016 Sep 22.

Mitochondrial diseases comprise a heterogeneous group of genetically inherited disorders that cause failures in energetic and metabolic function. Boosting residual oxidative phosphorylation (OXPHOS) activity can partially correct these failures. Herein, using a high-throughput chemical screen, we identified the bromodomain inhibitor I-BET 525762A as one of the top hits that increases COX5a protein levels in complex I (CI) mutant cybrid cells. In parallel, bromodomain-containing protein 4 (BRD4), a target of I-BET 525762A, was identified using a genome-wide CRISPR screen to search for genes whose loss of function rescues death of CI-impaired cybrids grown under conditions requiring OXPHOS activity for survival. We show that I-BET525762A or loss of

BRD4 remodeled the mitochondrial proteome to increase the levels and activity of OXPHOS protein complexes, leading to rescue of the bioenergetic defects and cell death caused by mutations or chemical inhibition of CI. These studies show that BRD4 inhibition may have therapeutic implications for the treatment of mitochondrial diseases.

Bezawork-Geleta, A., L. Dong, et al. "The Assembly Factor SDHAF2 Is Dispensable for Flavination of the Catalytic Subunit of Mitochondrial Complex II in Breast Cancer Cells." J Biol Chem. 2016 Oct 7;291(41):21414-21420. Epub 2016 Sep 1.

Mitochondrial complex II or succinate dehydrogenase (SDH) is at the crossroads of oxidative phosphorylation and the tricarboxylic acid cycle. It has been shown that Sdh5 (SDHAF2/SDH5 in mammals) is required for flavination of the subunit Sdh1 (SDHA in human cells) in yeast. Here we demonstrate that in human breast cancer cells, SDHAF2/SDH5 is dispensable for SDHA flavination. In contrast to yeast, CRISPR-Cas9 nickase-mediated SDHAF2 KO breast cancer cells feature flavinated SDHA and retain fully assembled and functional complex II, as well as normal mitochondrial respiration. Our data show that SDHA flavination is independent of SDHAF2 in breast cancer cells, employing an alternative mechanism.

Bhatt, M., C. Ivan, et al. "Drug-dependent functionalization of wild-type and mutant p53 in cisplatin-resistant human ovarian tumor cells." Oncotarget. 2016 Dec 26. doi: 10.18632/oncotarget.14228.

Cisplatin (cis-Pt) resistance in tumor cells from p53 dysfunction is a significant clinical problem. Although mutation can inhibit p53 function, >60% of p53 mutants retain normal function according to literature reports. Therefore, we examined the status of p53 in cisplatin-resistant ovarian tumor models and its functional response to cis-Pt and the mechanistically-distinct non-cross-resistant oxaliplatin (oxali-Pt). Relative to sensitive A2780 cells harboring wild-type p53, the 2780CP/CI-16, OVCAR-10, Hey and OVCA-433 cell lines were 10- to 30-fold resistant to cis-Pt, but was substantially circumvented by oxali-Pt. Mutant p53 in 2780CP/CI-16 (p53V172F) and OVCAR-10 (p53V172F and p53G266R) cells, predicted as non-functional in p53 database, displayed attenuated response to cis-Pt, as did the polymorphic p53P72R (functionally equivalent to wild-type p53) in HEY and OVCA-433 cell lines. However, p53 was robustly activated by oxali-Pt in all cell lines, with resultant drug potency confirmed as p53-dependent by p53 knockout using CRISPR/Cas9 system. This p53 activation by oxali-Pt was associated with phosphorylation at Ser20 by MEK1/2 based on

inhibitor and kinase studies. Cis-Pt, however, failed to phosphorylate Ser20 due to downregulated Chk2, and its clinical impact validated by reduced overall survival of ovarian cancer patients according to TCGA database. In conclusion, cis-Pt resistance occurs in both wild-type and mutant p53 ovarian cancer cells, but is associated with loss of Ser20 phosphorylation. However, these mutant p53, like polymorphic p53, are functional and activated by oxali-Pt-induced Ser20 phosphorylation. Thus, the potential exists for repurposing oxali-Pt or similar drugs against refractory cancers harboring wild-type or specific mutant p53.

Bialk, P., B. Sansbury, et al. "Analyses of point mutation repair and allelic heterogeneity generated by CRISPR/Cas9 and single-stranded DNA oligonucleotides." *Sci Rep*. 2016 Sep 9;6:32681. doi: [10.1038/srep32681](https://doi.org/10.1038/srep32681).

The repair of a point mutation can be facilitated by combined activity of a single-stranded oligonucleotide and a CRISPR/Cas9 system. While the mechanism of action of combinatorial gene editing remains to be elucidated, the regulatory circuitry of nucleotide exchange executed by oligonucleotides alone has been largely defined. The presence of the appropriate CRISPR/Cas9 system leads to an enhancement in the frequency of gene editing directed by single-stranded DNA oligonucleotides. While CRISPR/Cas9 executes double-stranded DNA cleavage efficiently, closure of the broken chromosomes is dynamic, as varying degrees of heterogeneity of the cleavage products appear to accompany the emergence of the corrected base pair. We provide a detailed analysis of allelic variance at and surrounding the target site. In one particular case, we report sequence alteration directed by a distinct member of the same gene family. Our data suggests that single-stranded DNA molecules may influence DNA junction heterogeneity created by CRISPR/Cas9.

Bluteau, D., J. Masliah-Planchon, et al. "Biallelic inactivation of REV7 is associated with Fanconi anemia." *J Clin Invest*. 2016 Sep 1;126(9):3580-4. doi: [10.1172/JCI88010](https://doi.org/10.1172/JCI88010). Epub 2016 Aug 8.

Fanconi anemia (FA) is a recessive genetic disease characterized by congenital abnormalities, chromosome instability, progressive bone marrow failure (BMF), and a strong predisposition to cancer. Twenty FA genes have been identified, and the FANC proteins they encode cooperate in a common pathway that regulates DNA crosslink repair and replication fork stability. We identified a child with severe BMF who harbored biallelic inactivating mutations of the translesion DNA synthesis (TLS) gene REV7 (also known as MAD2L2), which encodes the mutant REV7 protein REV7-V85E. Patient-derived cells

demonstrated an extended FA phenotype, which included increased chromosome breaks and G2/M accumulation upon exposure to DNA crosslinking agents, gammaH2AX and 53BP1 foci accumulation, and enhanced p53/p21 activation relative to cells derived from healthy patients. Expression of WT REV7 restored normal cellular and functional phenotypes in the patient's cells, and CRISPR/Cas9 inactivation of REV7 in a non-FA human cell line produced an FA phenotype. Finally, silencing Rev7 in primary hematopoietic cells impaired progenitor function, suggesting that the DNA repair defect underlies the development of BMF in FA. Taken together, our genetic and functional analyses identified REV7 as a previously undescribed FA gene, which we term FANCV.

Bompiani, K. M., C. Y. Tsai, et al. "Copper transporters and chaperones CTR1, CTR2, ATOX1, and CCS as determinants of cisplatin sensitivity." *Metallomics*. 2016 Sep 1;8(9):951-62. doi: [10.1039/c6mt00076b](https://doi.org/10.1039/c6mt00076b). Epub 2016 May 9.

The development of resistance to cisplatin (cDDP) is commonly accompanied by reduced drug uptake or increased efflux. Previous studies in yeast and murine embryonic fibroblasts have reported that the copper (Cu) transporters and chaperones participate in the uptake, efflux, and intracellular distribution of cDDP. However, there is conflicting data from studies in human cells. We used CRISPR-Cas9 genome editing to individually knock out the human copper transporters CTR1 and CTR2 and the copper chaperones ATOX1 and CCS. Isogenic knockout cell lines were generated in both human HEK-293T and ovarian carcinoma OVCAR8 cells. All knockout cell lines had slowed growth compared to parental cells, small changes in basal Cu levels, and varying sensitivities to Cu depending on the gene targeted. However, all of the knockouts demonstrated only modest 2 to 5-fold changes in cDDP sensitivity that did not differ from the range of sensitivities of 10 wild type clones grown from the same parental cell population. We conclude that, under basal conditions, loss of CTR1, CTR2, ATOX1, or CCS does not produce a change in cisplatin sensitivity that exceeds the variance found within the parental population, suggesting that they are not essential to the mechanism by which cDDP enters these cell lines and is transported to the nucleus.

Bonthron, D. T. and W. D. Foulkes "Genetics meets pathology - an increasingly important relationship." *J Pathol*. 2017 Jan;241(2):119-122. doi: [10.1002/path.4849](https://doi.org/10.1002/path.4849).

The analytical power of modern methods for DNA analysis has outstripped our capability to

interpret and understand the data generated. To make good use of this genomic data in a biomedical setting (whether for research or diagnosis), it is vital that we understand the mechanisms through which mutations affect biochemical pathways and physiological systems. This lies at the centre of what genetics is all about, and it is the reason why genetics and genomics should go hand in hand whenever possible. In this Annual Review Issue of *The Journal of Pathology*, we have assembled a collection of 16 expert reviews covering a wide range of topics. Through these, we illustrate the power of genetic analysis to improve our understanding of normal physiology and disease pathology, and thereby to think in rational ways about clinical management. Copyright (c) 2016 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

Cesar, S. A., V. Rajan, et al. "Insert, remove or replace: A highly advanced genome editing system using CRISPR/Cas9." *Biochim Biophys Acta*. 2016 Sep;1863(9):2333-44. doi: [10.1016/j.bbamer.2016.06.009](https://doi.org/10.1016/j.bbamer.2016.06.009). Epub 2016 Jun 24.

The clustered, regularly interspaced, short palindromic repeat (CRISPR) and CRISPR associated protein 9 (Cas9) system discovered as an adaptive immunity mechanism in prokaryotes has emerged as the most popular tool for the precise alterations of the genomes of diverse species. CRISPR/Cas9 system has taken the world of genome editing by storm in recent years. Its popularity as a tool for altering genomes is due to the ability of Cas9 protein to cause double-stranded breaks in DNA after binding with short guide RNA molecules, which can be produced with dramatically less effort and expense than required for production of transcription-activator like effector nucleases (TALEN) and zinc-finger nucleases (ZFN). This system has been exploited in many species from prokaryotes to higher animals including human cells as evidenced by the literature showing increasing sophistication and ease of CRISPR/Cas9 as well as increasing species variety where it is applicable. This technology is poised to solve several complex molecular biology problems faced in life science research including cancer research. In this review, we highlight the recent advancements in CRISPR/Cas9 system in editing genomes of prokaryotes, fungi, plants and animals and provide details on software tools available for convenient design of CRISPR/Cas9 targeting plasmids. We also discuss the future prospects of this advanced molecular technology.

Chandrasekharan, J. A., X. M. Huang, et al. "Altering the Anti-inflammatory Lipoxin Microenvironment: a New Insight into Kaposi's Sarcoma-Associated Herpesvirus Pathogenesis." *J Virol*. 2016 Nov

28;90(24):11020-11031. Print 2016 Dec 15.

Lipoxins are host anti-inflammatory molecules that play a vital role in restoring tissue homeostasis. The efficacy of lipoxins and their analog epilipoxins in treating inflammation and its associated diseases has been well documented. Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL) are two well-known inflammation related diseases caused by Kaposi's sarcoma-associated herpesvirus (KSHV). Controlling inflammation is one of the strategies adopted to treat KS and PEL, a primary motivation for exploring and evaluating the therapeutic potential of using lipoxins. This study documents how KSHV manipulates and downregulates the secretion of the anti-inflammatory lipoxin A4 in host cells and the viral factors involved in this process using in vitro KS and PEL cells as models. The presence of the lipoxin A4 receptor/formyl peptidyl receptor (ALX/FPR) in KS patient tissue sections and in vitro KS and PEL cell models offers a novel possibility for treating KS and PEL with lipoxins. Treating de novo KSHV-infected endothelial cells with lipoxin and epilipoxin creates an anti-inflammatory environment by decreasing the levels of NF-kappaB, AKT, ERK1/2, COX-2, and 5-lipoxygenase. Lipoxin treatment on CRISPR/CAS9 technology-mediated ALX/FPR gene deletion revealed the importance of the lipoxin receptor ALX for effective lipoxin signaling. A viral microRNA (miRNA) cluster was identified as the primary factor contributing to the downregulation of lipoxin A4 secretion in host cells. The KSHV miRNA cluster probably targets enzyme 15-lipoxygenase, which is involved in lipoxin A4 synthesis. This study provides a new insight into the potential treatment of KS and PEL using nature's own anti-inflammatory molecule, lipoxin. **IMPORTANCE:** KSHV infection has been shown to upregulate several host proinflammatory factors, which aid in its survival and pathogenesis. The influence of KSHV infection on anti-inflammatory molecules is not well studied. Since current treatment methods for KS and PEL are fraught with unwanted side effects and low efficiency, the search for new therapeutics is therefore imperative. The use of nature's own molecule lipoxin as a drug is promising. This study opens up new domains in KSHV research focusing on how the virus modulates lipoxin secretion and warrants further investigation of the therapeutic potential of lipoxin using in vitro cell models for KS and PEL.

Chen, B., J. Liu, et al. "ERK-mediated NF-kappaB activation through ASIC1 in response to acidosis." *Oncogenesis*. 2016 Dec 12;5(12):e279. doi: [10.1038/onesis.2016.81](https://doi.org/10.1038/onesis.2016.81).

Acidic microenvironment is a common feature of solid tumors. We have previously shown

that neuron specific acid-sensing ion channel 1 (ASIC1) is expressed in breast cancer, and it is responsible for acidosis-induced cellular signaling through AKT, leading to nuclear factor-kappaB (NF-kappaB) activation, and cell invasion and metastasis. However, AKT is frequently activated in cancer. Thus, a key question is whether ASIC1-mediated cell signaling still takes place in the cancer cells carrying constitutively active AKT. In the present study, we show that among four prostate cancer cell lines tested, 22Rv1 cells express the highest level of phosphorylated AKT that is not impacted by acidosis. However, acidosis can still induce NF-kappaB activation during which extracellular signal-regulated kinase (ERK) serves as an alternative pathway for ASIC-mediated cell signaling. Inhibition of ERK by chemical inhibitors or small interfering RNAs suppresses the acidosis-induced NF-kappaB activity through regulation of the inhibitory subunit IkbppaB phosphorylation. Furthermore, suppression of ASIC1-mediated generation of reactive oxygen species (ROS) by ROS scavengers, such as glutathione or N-acetyl-cysteine causes a decrease in ERK phosphorylation and degradation of IkbppaB. Finally, ASIC1 is upregulated in a subset of prostate cancer cases and ASIC1 knockout by CRISPR/Cas9 significantly suppresses cell invasion, and castration resistance both in vitro and in vivo. Together, these results support the significance of ASIC1-ROS-ERK-IkbppaB-NF-kappaB axis in prostate tumorigenesis, especially in the constitutively active AKT background.

Cho, H. and W. G. Kaelin "Targeting HIF2 in Clear Cell Renal Cell Carcinoma." Cold Spring Harb Symp Quant Biol. 2016 Dec 8. pii: 030833.

Inactivation of the von Hippel-Lindau tumor-suppressor protein (pVHL) is the signature "truncal" event in clear cell renal cell carcinoma, which is the most common form of kidney cancer. pVHL is part of a ubiquitin ligase that targets the alpha subunit of the hypoxia-inducible factor (HIF) transcription factor for destruction when oxygen is available. Preclinical studies strongly suggest that deregulation of HIF, and particularly HIF2, drives pVHL-defective renal carcinogenesis. Although HIF2alpha was classically considered undruggable, structural and chemical work by Rick Bruick and Kevin Gardner at University of Texas Southwestern laid the foundation for the development of small molecule direct HIF2alpha antagonists (PT2385 and the related tool compound PT2399) by Peloton Therapeutics that block the dimerization of HIF2alpha with its partner protein ARNT1. These compounds inhibit clear cell renal cell carcinoma growth in preclinical models, and PT2385 has now entered the clinic. Nonetheless, the

availability of such compounds, together with clustered regularly interspaced short palindromic repeat (CRISPR)-based gene editing approaches, has revealed a previously unappreciated heterogeneity among clear cell renal carcinomas and patient-derived xenografts with respect to HIF2 dependence, suggesting that predictive biomarkers will be needed to optimize the use of such agents in the clinic.

Chung, J., S. Aburaya, et al. "Molecular changes in appearance of a cancer cell among normal HEK293T cells." J Biosci Bioeng. 2016 Oct 21. pii: S1389-1723(16)30329-2. doi: 10.1016/j.jbiosc.2016.09.012.

In very early stages of cancer development, one or a few cells expressing cancer-associated genes appear among a much larger number of surrounding normal cells. To analyze the molecular changes induced by this co-existence, we artificially prepared transformed cells with complete loss of tumor suppressor gene, SCRIB, among normal human embryonic kidney (HEK293T) cells. A cell strain with SCRIB-knockout was successfully constructed by using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 nuclease system and co-cultured with normal cells. By measuring the time-course changes in cell numbers when SCRIB-knockout cells (cancer model) or cells with normal level of SCRIB expression were respectively co-cultured with wild-type normal HEK293T cells, it was shown that the SCRIB-knockout strain was beneficial for proliferation when mixed together with normal cells. Moreover, as a result of proteome analysis on wild-type cells separated from co-culture with SCRIB-knockout cells, a total of 843 proteins were identified, among which 139 proteins were specific. Among the specifically identified proteins, 22 proteins were annotated to be involved in cytoskeletons including microtubule motor activity-associated proteins. It was implied that molecular changes in cytoskeletons occurred in normal cells when co-cultured with SCRIB knockout cells, but the SCRIB knockout might affect proliferation of the transformed cells with SCRIB knockout by defensive or offensive mechanism of surrounding normal cells.

Cockrell, A. S., B. L. Yount, et al. "A mouse model for MERS coronavirus-induced acute respiratory distress syndrome." Nat Microbiol. 2016 Nov 28;2:16226. doi: 10.1038/nmicrobiol.2016.226.

Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel virus that emerged in 2012, causing acute respiratory distress syndrome (ARDS), severe pneumonia-like symptoms and multi-organ failure, with a case fatality rate of approximately 36%. Limited clinical studies indicate that humans infected with MERS-CoV exhibit

pathology consistent with the late stages of ARDS, which is reminiscent of the disease observed in patients infected with severe acute respiratory syndrome coronavirus. Models of MERS-CoV-induced severe respiratory disease have been difficult to achieve, and small-animal models traditionally used to investigate viral pathogenesis (mouse, hamster, guinea-pig and ferret) are naturally resistant to MERS-CoV. Therefore, we used CRISPR-Cas9 gene editing to modify the mouse genome to encode two amino acids (positions 288 and 330) that match the human sequence in the dipeptidyl peptidase 4 receptor, making mice susceptible to MERS-CoV infection and replication. Serial MERS-CoV passage in these engineered mice was then used to generate a mouse-adapted virus that replicated efficiently within the lungs and evoked symptoms indicative of severe ARDS, including decreased survival, extreme weight loss, decreased pulmonary function, pulmonary haemorrhage and pathological signs indicative of end-stage lung disease. Importantly, therapeutic countermeasures comprising MERS-CoV neutralizing antibody treatment or a MERS-CoV spike protein vaccine protected the engineered mice against MERS-CoV-induced ARDS.

Czarnek, M. and J. Bereta "The CRISPR-Cas system - from bacterial immunity to genome engineering." Postepy Hig Med Dosw (Online). 2016 Sep 1;70(0):901-16.

Precise and efficient genome modifications present a great value in attempts to comprehend the roles of particular genes and other genetic elements in biological processes as well as in various pathologies. In recent years novel methods of genome modification known as genome editing, which utilize so called "programmable" nucleases, came into use. A true revolution in genome editing has been brought about by the introduction of the CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR associated) system, in which one of such nucleases, i.e. Cas9, plays a major role. This system is based on the elements of the bacterial and archaeal mechanism responsible for acquired immunity against phage infections and transfer of foreign genetic material. Microorganisms incorporate fragments of foreign DNA into CRISPR loci present in their genomes, which enables fast recognition and elimination of future infections. There are several types of CRISPR-Cas systems among prokaryotes but only elements of CRISPR type II are employed in genome engineering. CRISPR-Cas type II utilizes small RNA molecules (crRNA and tracrRNA) to precisely direct the effector nuclease - Cas9 - to a specific site in the genome, i.e. to the sequence complementary to crRNA. Cas9 may be used to: (i)

introduce stable changes into genomes e.g. in the process of generation of knock-out and knock-in animals and cell lines, (ii) activate or silence the expression of a gene of interest, and (iii) visualize specific sites in genomes of living cells. The CRISPR-Cas-based tools have been successfully employed for generation of animal and cell models of a number of diseases, e.g. specific types of cancer. In the future, the genome editing by programmable nucleases may find wide application in medicine e.g. in the therapies of certain diseases of genetic origin and in the therapy of HIV-infected patients.

De Ravin, S. S., L. Li, et al. "CRISPR-Cas9 gene repair of hematopoietic stem cells from patients with X-linked chronic granulomatous disease." Sci Transl Med. 2017 Jan 11;9(372). pii: eaah3480. doi: 10.1126/scitranslmed.aah3480.

Gene repair of CD34+ hematopoietic stem and progenitor cells (HSPCs) may avoid problems associated with gene therapy, such as vector-related mutagenesis and dysregulated transgene expression. We used CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 (CRISPR-associated 9) to repair a mutation in the CYBB gene of CD34+ HSPCs from patients with the immunodeficiency disorder X-linked chronic granulomatous disease (X-CGD). Sequence-confirmed repair of >20% of HSPCs from X-CGD patients restored the function of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase and superoxide radical production in myeloid cells differentiated from these progenitor cells in vitro. Transplant of gene-repaired X-CGD HSPCs into NOD (nonobese diabetic) SCID (severe combined immunodeficient) gammac^{-/-} mice resulted in efficient engraftment and production of functional mature human myeloid and lymphoid cells for up to 5 months. Whole-exome sequencing detected no indels outside of the CYBB gene after gene correction. CRISPR-mediated gene editing of HSPCs may be applicable to other CGD mutations and other monogenic disorders of the hematopoietic system.

Dever, D. P., R. O. Bak, et al. "CRISPR/Cas9 beta-globin gene targeting in human haematopoietic stem cells." Nature. 2016 Nov 17;539(7629):384-389. doi: 10.1038/nature20134. Epub 2016 Nov 7.

The beta-haemoglobinopathies, such as sickle cell disease and beta-thalassaemia, are caused by mutations in the beta-globin (HBB) gene and affect millions of people worldwide. Ex vivo gene correction in patient-derived haematopoietic stem cells followed by autologous transplantation could be used to cure beta-haemoglobinopathies. Here we present a CRISPR/Cas9 gene-editing system that combines Cas9 ribonucleoproteins and adeno-associated viral vector

delivery of a homologous donor to achieve homologous recombination at the HBB gene in haematopoietic stem cells. Notably, we devise an enrichment model to purify a population of haematopoietic stem and progenitor cells with more than 90% targeted integration. We also show efficient correction of the Glu6Val mutation responsible for sickle cell disease by using patient-derived stem and progenitor cells that, after differentiation into erythrocytes, express adult beta-globin (HbA) messenger RNA, which confirms intact transcriptional regulation of edited HBB alleles. Collectively, these preclinical studies outline a CRISPR-based methodology for targeting haematopoietic stem cells by homologous recombination at the HBB locus to advance the development of next-generation therapies for beta-haemoglobinopathies.

Dolatshad, H., A. Pellagatti, et al. "Cryptic splicing events in the iron transporter ABCB7 and other key target genes in SF3B1-mutant myelodysplastic syndromes." Leukemia. 2016 Dec;30(12):2322-2331. doi: 10.1038/leu.2016.149. Epub 2016 May 23.

The splicing factor SF3B1 is the most frequently mutated gene in myelodysplastic syndromes (MDS), and is strongly associated with the presence of ring sideroblasts (RS). We have performed a systematic analysis of cryptic splicing abnormalities from RNA sequencing data on hematopoietic stem cells (HSCs) of SF3B1-mutant MDS cases with RS. Aberrant splicing events in many downstream target genes were identified and cryptic 3' splice site usage was a frequent event in SF3B1-mutant MDS. The iron transporter ABCB7 is a well-recognized candidate gene showing marked downregulation in MDS with RS. Our analysis unveiled aberrant ABCB7 splicing, due to usage of an alternative 3' splice site in MDS patient samples, giving rise to a premature termination codon in the ABCB7 mRNA. Treatment of cultured SF3B1-mutant MDS erythroblasts and a CRISPR/Cas9-generated SF3B1-mutant cell line with the nonsense-mediated decay (NMD) inhibitor cycloheximide showed that the aberrantly spliced ABCB7 transcript is targeted by NMD. We describe cryptic splicing events in the HSCs of SF3B1-mutant MDS, and our data support a model in which NMD-induced downregulation of the iron exporter ABCB7 mRNA transcript resulting from aberrant splicing caused by mutant SF3B1 underlies the increased mitochondrial iron accumulation found in MDS patients with RS.

Doll, S., B. Proneth, et al. "ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition." Nat Chem Biol. 2017 Jan;13(1):91-98. doi: 10.1038/nchembio.2239. Epub 2016 Nov 14.

Ferroptosis is a form of regulated necrotic cell death controlled by glutathione peroxidase 4 (GPX4). At present, mechanisms that could predict sensitivity and/or resistance and that may be exploited to modulate ferroptosis are needed. We applied two independent approaches—a genome-wide CRISPR-based genetic screen and microarray analysis of ferroptosis-resistant cell lines—to uncover acyl-CoA synthetase long-chain family member 4 (ACSL4) as an essential component for ferroptosis execution. Specifically, Gpx4-Acsl4 double-knockout cells showed marked resistance to ferroptosis. Mechanistically, ACSL4 enriched cellular membranes with long polyunsaturated omega6 fatty acids. Moreover, ACSL4 was preferentially expressed in a panel of basal-like breast cancer cell lines and predicted their sensitivity to ferroptosis. Pharmacological targeting of ACSL4 with thiazolidinediones, a class of antidiabetic compound, ameliorated tissue demise in a mouse model of ferroptosis, suggesting that ACSL4 inhibition is a viable therapeutic approach to preventing ferroptosis-related diseases.

Dong, Z., M. Huang, et al. "Focused screening of mitochondrial metabolism reveals a crucial role for a tumor suppressor Hbp1 in ovarian reserve." Cell Death Differ. 2016 Oct;23(10):1602-14. doi: 10.1038/cdd.2016.47. Epub 2016 May 20.

Granulosa cells (GCs) are tightly associated with fertility and the fate of ovarian follicles. Mitochondria are the central executors of apoptosis. However, the genetic basis underlying mitochondrial modulation in GCs during the ovarian development is poorly understood. Here, CRISPR/Cas9-mediated genetic screening was used to identify genes conferring mitochondrial metabolism in human GCs. The results uncovered roles for several tumor suppressors, including HBP1, in the augmentation of mitochondrial function. Focused analysis revealed that high-mobility group (HMG)-box transcription factor 1 (Hbp1) levels regulate mitochondrial biogenesis, which is associated with global changes in transcription including Tfam. The systemic or granulosa-specific but not oocyte-specific ablation of Hbp1 promoted follicle growth and oocyte production, and is associated with the reduced apoptotic signals in mouse GCs. Consistent with increased mitochondrial function and attenuated GC apoptosis, the regulation of Hbp1 conferred substantial protection of ovarian reserve. Thus, the results of the present study provide a critical target to understand the control of the reproductive lifespan.

Duan, Y. and H. Fang "RecQL4 regulates autophagy and apoptosis in U2OS cells." Biochem Cell Biol.

2016 Dec;94(6):551-559. Epub 2016 May 3.

RecQL4, one of the 5 human RecQ helicases, is a key mediator of genomic stability and its deficiency can cause premature aging phenotypes. Here, by using CRISPR/Cas and RNAi technology, we demonstrated that autophagy level was elevated in both RecQL4 knockdown and knockout cells compared with those of the control cells. Surprisingly, mitochondrial content was increased and LC3 colocalization with mitochondria was partially lost in RecQL4 knockout cells compared with the control cells, suggesting that RecQL4 deficiency impaired mitophagic processes in U2OS cells. Furthermore, we found that knockout of RecQL4 destabilized PINK1. In addition, RecQL4 knockout cells were more susceptible to apoptosis under mitochondrial stress than the control cells. In conclusion, our findings indicated a novel role of RecQL4 in the regulation of autophagy/mitophagy in U2OS cells.

Dubey, R., A. M. Lebensohn, et al. "Chromatin-Remodeling Complex SWI/SNF Controls Multidrug Resistance by Transcriptionally Regulating the Drug Efflux Pump ABCB1." Cancer Res. 2016 Oct 1;76(19):5810-5821. Epub 2016 Aug 8.

Anthracyclines are among the most effective yet most toxic drugs used in the oncology clinic. The nucleosome-remodeling SWI/SNF complex, a potent tumor suppressor, is thought to promote sensitivity to anthracyclines by recruiting topoisomerase IIa (TOP2A) to DNA and increasing double-strand breaks. In this study, we discovered a novel mechanism through which SWI/SNF influences resistance to the widely used anthracycline doxorubicin based on the use of a forward genetic screen in haploid human cells, followed by a rigorous single and double-mutant epistasis analysis using CRISPR/Cas9-mediated engineering. Doxorubicin resistance conferred by loss of the SMARCB1 subunit of the SWI/SNF complex was caused by transcriptional upregulation of a single gene, encoding the multidrug resistance pump ABCB1. Remarkably, both ABCB1 upregulation and doxorubicin resistance caused by SMARCB1 loss were dependent on the function of SMARCA4, a catalytic subunit of the SWI/SNF complex. We propose that residual SWI/SNF complexes lacking SMARCB1 are vital determinants of drug sensitivity, not just to TOP2A-targeted agents, but to the much broader range of cancer drugs effluxed by ABCB1. Cancer Res; 76(19); 5810-21. (c)2016 AACR.

Fachinetti, D., G. A. Logsdon, et al. "CENP-A Modifications on Ser68 and Lys124 Are Dispensable for Establishment, Maintenance, and Long-Term Function of Human Centromeres." Dev Cell. 2017 Jan 9;40(1):104-113. doi: 10.1016/j.devcel.2016.12.014.

CENP-A is a histone H3 variant key to epigenetic specification of mammalian centromeres. Using transient overexpression of CENP-A mutants, two recent reports in *Developmental Cell* proposed essential centromere functions for post-translational modifications of human CENP-A. Phosphorylation at Ser68 was proposed to have an essential role in CENP-A deposition at centromeres. Blockage of ubiquitination at Lys124 was proposed to abrogate localization of CENP-A to the centromere. Following gene inactivation and replacement in human cells, we demonstrate that CENP-A mutants that cannot be phosphorylated at Ser68 or ubiquitinated at Lys124 assemble efficiently at centromeres during G1, mediate early events in centromere establishment at an ectopic chromosomal locus, and maintain centromere function indefinitely. Thus, neither Ser68 nor Lys124 post-translational modification is essential for long-term centromere identity, propagation, cell-cycle-dependent deposition, maintenance, function, or mediation of early steps in centromere establishment.

Fellmann, C., B. G. Gowen, et al. "Cornerstones of CRISPR-Cas in drug discovery and therapy." Nat Rev Drug Discov. 2016 Dec 23. doi: 10.1038/nrd.2016.238.

The recent development of CRISPR-Cas systems as easily accessible and programmable tools for genome editing and regulation is spurring a revolution in biology. Paired with the rapid expansion of reference and personalized genomic sequence information, technologies based on CRISPR-Cas are enabling nearly unlimited genetic manipulation, even in previously difficult contexts, including human cells. Although much attention has focused on the potential of CRISPR-Cas to cure Mendelian diseases, the technology also holds promise to transform the development of therapies to treat complex heritable and somatic disorders. In this Review, we discuss how CRISPR-Cas can affect the next generation of drugs by accelerating the identification and validation of high-value targets, uncovering high-confidence biomarkers and developing differentiated breakthrough therapies. We focus on the promises, pitfalls and hurdles of this revolutionary gene-editing technology, discuss key aspects of different CRISPR-Cas screening platforms and offer our perspectives on the best practices in genome engineering.

Feng, J., J. Jing, et al. "Generation and characterization of tamoxifen-inducible Pax9-CreER knock-in mice using CrispR/Cas9." Genesis. 2016 Sep;54(9):490-6. doi: 10.1002/dvg.22956. Epub 2016 Jul 26.

Pax9 encodes a paired-box homeodomain (Pax) transcription factor and is critical for the development of multiple organs. Using CrispR/Cas9-mediated homologous directed repair (HDR), we

generated a new Pax9-CreER knock-in mouse line in which the CreER(T2) fusion protein is produced after synthesis of endogenous Pax9 protein. We found that tdTomato reporter expression in Pax9-CreER;tdTomato reporter mice is detectable in a similar pattern to the endogenous Pax9 expression, faithfully recapitulating the Pax9 expression domains throughout the embryo and in the adult mouse. At early embryonic stages, the tdTomato reporter is expressed first in the pharyngeal pouch region and later in the craniofacial mesenchyme, somites, limbs, and lingual papillae in the adult tongue. These results demonstrate that this new Pax9-CreER knock-in mouse line can be used for lineage tracing and genetic targeting of Pax9-expressing cells and their progeny in a temporally and spatially controlled manner during development and organogenesis.

Flahaut, M., N. Jauquier, et al. "Aldehyde dehydrogenase activity plays a Key role in the aggressive phenotype of neuroblastoma." *BMC Cancer*. 2016 Oct 10;16(1):781.

BACKGROUND: The successful targeting of neuroblastoma (NB) by associating tumor-initiating cells (TICs) is a major challenge in the development of new therapeutic strategies. The subfamily of aldehyde dehydrogenases 1 (ALDH1) isoenzymes, which comprises ALDH1A1, ALDH1A2, and ALDH1A3, is involved in the synthesis of retinoic acid, and has been identified as functional stem cell markers in diverse cancers. By combining serial neurosphere passages with gene expression profiling, we have previously identified ALDH1A2 and ALDH1A3 as potential NB TICs markers in patient-derived xenograft tumors. In this study, we explored the involvement of ALDH1 isoenzymes and the related ALDH activity in NB aggressive properties. **METHODS:** ALDH activity and ALDH1A1/A2/A3 expression levels were measured using the ALDEFLUOR kit, and by real-time PCR, respectively. ALDH activity was inhibited using the specific ALDH inhibitor diethylaminobenzaldehyde (DEAB), and ALDH1A3 gene knock-out was generated through the CRISPR/Cas9 technology. **RESULTS:** We first confirmed the enrichment of ALDH1A2 and ALDH1A3 mRNA expression in NB cell lines and patient-derived xenograft tumors during neurosphere passages. We found that high ALDH1A1 expression was associated with less aggressive NB tumors and cell lines, and correlated with favorable prognostic factors. In contrast, we observed that ALDH1A3 was more widely expressed in NB cell lines and was associated with poor survival and high-risk prognostic factors. We also identified an important ALDH activity in various NB cell lines and patient-derived xenograft tumors. Specific inhibition of ALDH activity with diethylaminobenzaldehyde (DEAB)

resulted in a strong reduction of NB cell clonogenicity, and TIC self-renewal potential, and partially enhanced NB cells sensitivity to 4-hydroxycyclophosphamide. Finally, the specific knock-out of ALDH1A3 via CRISPR/Cas9 gene editing reduced NB cell clonogenicity, and mediated a cell type-dependent inhibition of TIC self-renewal properties. **CONCLUSIONS:** Together our data uncover the participation of ALDH enzymatic activity in the aggressive properties and 4-hydroxycyclophosphamide resistance of NB, and show that the specific ALDH1A3 isoenzyme increases the aggressive capacities of a subset of NB cells.

Fletcher, N. M., J. Belotte, et al. "Specific point mutations in key redox enzymes are associated with chemoresistance in epithelial ovarian cancer." *Free Radic Biol Med*. 2017 Jan;102:122-132. doi: [10.1016/j.freeradbiomed.2016.11.028](https://doi.org/10.1016/j.freeradbiomed.2016.11.028). Epub 2016 Nov 25.

Oxidative stress plays an important role in the pathophysiology of ovarian cancer. Resistance to chemotherapy presents a significant challenge for ovarian cancer treatment. Specific single nucleotide polymorphisms (SNPs) in key redox enzymes have been associated with ovarian cancer survival and progression. The objective of this study was to determine whether chemotherapy induces point mutations in key redox enzymes that lead to the acquisition of chemoresistance in epithelial ovarian cancer (EOC). Human EOC cell lines and their chemoresistant counterpart were utilized for this study. Specific SNPs in key redox enzymes were analyzed by TaqMan SNP Genotyping. Activities and levels of key redox enzymes were determined by real-time RT-PCR, ELISA and a greiss assay. Point mutations in key redox enzymes were introduced into sensitive EOC cells via the CRISPR/Cas9 system. Cell viability and IC50 for cisplatin were determined by the MTT Cell Proliferation Assay. Data was analyzed with SPSS using Student's two-tailed t-tests and One-way ANOVA followed by Dunnett's or Tukey's post hoc tests, $p < 0.05$. Here, we demonstrate that chemoresistant EOC cells are characterized by a further enhancement in oxidative stress as compared to sensitive counterparts. Additionally, chemoresistant EOC cells manifested specific point mutations, which are associated with altered enzymatic activity, in key redox enzymes that are not detected in sensitive counterparts. Supplementation of an antioxidant was able to successfully sensitize EOC cells to chemotherapeutics. Causality was established by the induction of these point mutations in sensitive EOC cells, which resulted in a significant increase in the level of chemoresistance. These findings indicate that chemotherapy induces specific point mutations in key

redox enzymes that contribute to the acquisition of chemoresistance in EOC cells, highlighting a potential novel mechanism. Identification of targets for chemoresistance with either biomarker and/or screening potential will have a significant impact for the treatment of this disease.

Fregoso, O. I. and M. Emerman "Activation of the DNA Damage Response Is a Conserved Function of HIV-1 and HIV-2 Vpr That Is Independent of SLX4 Recruitment." *MBio*. 2016 Sep 13;7(5). pii: e01433-16. doi: [10.1128/mBio.01433-16](https://doi.org/10.1128/mBio.01433-16).

There has been extraordinary progress in understanding the roles of lentiviral accessory proteins in antagonizing host antiviral defense proteins. However, the precise primary function of the accessory gene Vpr remains elusive. Here we suggest that engagement with the DNA damage response is an important function of primate lentiviral Vpr proteins because of its conserved function among diverse lentiviral lineages. In contrast, we show that, for HIV-1, HIV-2, and related Vpr isolates and orthologs, there is a lack of correlation between DNA damage response activation and interaction with the host SLX4 protein complex of structure specific endonucleases; some Vpr proteins are able to interact with SLX4, but the majority are not. Using the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 method to knock out SLX4, we formally showed that HIV-1 and HIV-2 Vpr orthologs can still activate the DNA damage response and cell cycle arrest in the absence of SLX4. Together, our data suggest that activation of the DNA damage response, but not SLX4 interaction, is conserved and therefore indicative of an important function of Vpr. Our data also indicate that Vpr activates the DNA damage response through an SLX4-independent mechanism that remains uncharacterized. **IMPORTANCE:** HIV-1 and HIV-2 belong to a family of viruses called lentiviruses that infect at least 40 primate species, including humans. Lentiviruses have been circulating in primates for at least 5 million years. In order to better fight HIV, we must understand the viral and host factors necessary for infection, adaptation, and transmission of these viruses. Using the natural variation of HIV-1, HIV-2, and related lentiviruses, we have investigated the role of the DNA damage response in the viral life cycle. We have found that the ability of lentiviruses to activate the DNA damage response is largely conserved. However, we also found that the SLX4 host factor is not required for this activation, as was previously proposed. This indicates that the DNA damage response is an important player in the viral life cycle, and yet the mechanism(s) by which HIV-1, HIV-2, and other primate lentiviruses engage the DNA damage response is still unknown.

Gao, L., X. Wang, et al. "FGF19/FGFR4 signaling contributes to the resistance of hepatocellular carcinoma to sorafenib." *J Exp Clin Cancer Res*. 2017 Jan 9;36(1):8. doi: [10.1186/s13046-016-0478-9](https://doi.org/10.1186/s13046-016-0478-9).

BACKGROUND: Sorafenib, a multi-kinase inhibitor, is used as a standard therapy for advanced hepatocellular carcinoma (HCC). However, complete remission has not been achieved and the molecular basis of HCC resistance to sorafenib remains largely unknown. Previous studies have shown that fibroblast growth factor 19 (FGF19) expression correlates with tumor progression and poor prognosis of HCC. Here, we demonstrate the novel role of FGF19 in HCC resistance to sorafenib therapy. **METHODS:** FGF19 Knockdown cells were achieved by lentiviral-mediated interference, and FGFR4 knockout cells were achieved by CRISPR-Cas9. Protein levels of FGF19, FGFR4 and c-PARP in various HCC cell lines were measured by Western blotting analysis. Cell viability was determined by MTS assay, apoptosis was determined by DAPI nuclear staining and Western blot of c-PRAP, and ROS generation was determined by DCFH-DA staining and electrochemical biosensor. **RESULTS:** We showed that FGF19, when overexpressed, inhibited the effect of sorafenib on ROS generation and apoptosis in HCC. In contrast, loss of FGF19 or its receptor FGFR4 led to a remarkable increase in sorafenib-induced ROS generation and apoptosis. In addition, knockdown of FGF19 in sorafenib-resistant HCC cells significantly enhanced the sensitivity to sorafenib. Importantly, targeting FGF19/FGFR4 axis by ponatinib, a third-generation inhibitor of chronic myeloid leukemia, overcomes HCC resistance of sorafenib by enhancing ROS-associated apoptosis in sorafenib-treated HCC. **CONCLUSION:** Our results provide the first evidence that inhibition of FGF19/FGFR4 signaling significantly overcomes sorafenib resistance in HCC. Co-treatment of ponatinib and sorafenib may represent an effective therapeutic approach for eradicating HCC.

Gao, Y., X. Xiong, et al. "Complex transcriptional modulation with orthogonal and inducible dCas9 regulators." *Nat Methods*. 2016 Dec;13(12):1043-1049. doi: [10.1038/nmeth.4042](https://doi.org/10.1038/nmeth.4042). Epub 2016 Oct 24.

The ability to dynamically manipulate the transcriptome is important for studying how gene networks direct cellular functions and how network perturbations cause disease. Nuclease-dead CRISPR-dCas9 transcriptional regulators, while offering an approach for controlling individual gene expression, remain incapable of dynamically coordinating complex transcriptional events. Here, we describe a flexible dCas9-based platform for chemical-inducible complex gene regulation. From a screen of chemical- and light-inducible dimerization systems, we identified

two potent chemical inducers that mediate efficient gene activation and repression in mammalian cells. We combined these inducers with orthogonal dCas9 regulators to independently control expression of different genes within the same cell. Using this platform, we further devised AND, OR, NAND, and NOR dCas9 logic operators and a diametric regulator that activates gene expression with one inducer and represses with another. This work provides a robust CRISPR-dCas9-based platform for enacting complex transcription programs that is suitable for large-scale transcriptome engineering.

Garcia-Bloj, B., C. Moses, et al. "Waking up dormant tumor suppressor genes with zinc fingers, TALEs and the CRISPR/dCas9 system." *Oncotarget*. 2016 Sep 13;7(37):60535-60554. doi: 10.18632/oncotarget.11142.

The aberrant epigenetic silencing of tumor suppressor genes (TSGs) plays a major role during carcinogenesis and regaining these dormant functions by engineering of sequence-specific epigenome editing tools offers a unique opportunity for targeted therapies. However, effectively normalizing the expression and regaining tumor suppressive functions of silenced TSGs by artificial transcription factors (ATFs) still remains a major challenge. Herein we describe novel combinatorial strategies for the potent reactivation of two class II TSGs, MASPIN and REPRIMO, in cell lines with varying epigenetic states, using the CRISPR/dCas9 associated system linked to a panel of effector domains (VP64, p300, VPR and SAM complex), as well as with protein-based ATFs, Zinc Fingers and TALEs. We found that co-delivery of multiple effector domains using a combination of CRISPR/dCas9 and TALEs or SAM complex maximized activation in highly methylated promoters. In particular, CRISPR/dCas9 VPR with SAM upregulated MASPIN mRNA (22,145-fold change) in H157 lung cancer cells, with accompanying re-expression of MASPIN protein, which led to a concomitant inhibition of cell proliferation and induction of apoptotic cell death. Consistently, CRISPR/dCas9 VP64 with SAM upregulated REPRIMO (680-fold change), which led to phenotypic reprogramming in AGS gastric cancer cells. Altogether, our results outlined novel sequence-specific, combinatorial epigenome editing approaches to reactivate highly methylated TSGs as a promising therapy for cancer and other diseases.

Ge, D. T., C. Tipping, et al. "Rapid Screening for CRISPR-Directed Editing of the Drosophila Genome Using white Coconversion." *G3 (Bethesda)*. 2016 Oct 13;6(10):3197-3206. doi: 10.1534/g3.116.032557.

Adoption of a streamlined version of the

bacterial clustered regular interspersed short palindromic repeat (CRISPR)/Cas9 defense system has accelerated targeted genome engineering. The *Streptococcus pyogenes* Cas9 protein, directed by a simplified, CRISPR-like single-guide RNA, catalyzes a double-stranded DNA break at a specific genomic site; subsequent repair by end joining can introduce mutagenic insertions or deletions, while repair by homologous recombination using an exogenous DNA template can incorporate new sequences at the target locus. However, the efficiency of Cas9-directed mutagenesis is low in *Drosophila melanogaster*. Here, we describe a strategy that reduces the time and effort required to identify flies with targeted genomic changes. The strategy uses editing of the white gene, evidenced by altered eye color, to predict successful editing of an unrelated gene-of-interest. The red eyes of wild-type flies are readily distinguished from white-eyed (end-joining-mediated loss of White function) or brown-eyed (recombination-mediated conversion to the whitecoffee allele) mutant flies. When single injected G0 flies produce individual G1 broods, flies carrying edits at a gene-of-interest were readily found in broods in which all G1 offspring carried white mutations. Thus, visual assessment of eye color substitutes for wholesale PCR screening of large numbers of G1 offspring. We find that end-joining-mediated mutations often show signatures of microhomology-mediated repair and that recombination-based mutations frequently involve donor plasmid integration at the target locus. Finally, we show that gap repair induced by two guide RNAs more reliably converts the intervening target sequence, whereas the use of Lig4169 mutants to suppress end joining does not improve recombination efficacy.

Goyal, A., K. Myacheva, et al. "Challenges of CRISPR/Cas9 applications for long non-coding RNA genes." *Nucleic Acids Res*. 2016 Sep 30. pii: gkw883.

The CRISPR/Cas9 system provides a revolutionary genome editing tool for all areas of molecular biology. In long non-coding RNA (lncRNA) research, the Cas9 nuclease can delete lncRNA genes or introduce RNA-destabilizing elements into their locus. The nuclease-deficient dCas9 mutant retains its RNA-dependent DNA-binding activity and can modulate gene expression when fused to transcriptional repressor or activator domains. Here, we systematically analyze whether CRISPR approaches are suitable to target lncRNAs. Many lncRNAs are derived from bidirectional promoters or overlap with promoters or bodies of sense or antisense genes. In a genome-wide analysis, we find only 38% of 15929 lncRNA loci are safely amenable to CRISPR applications while almost two-thirds of lncRNA loci are at risk to inadvertently deregulate neighboring

genes. CRISPR- but not siPOOL or Antisense Oligo (ASO)-mediated targeting of lncRNAs NOP14-AS1, LOC389641, MNX1-AS1 or HOTAIR also affects their respective neighboring genes. Frequently overlooked, the same restrictions may apply to mRNAs. For example, the tumor suppressor TP53 and its head-to-head neighbor WRAP53 are jointly affected by the same sgRNAs but not siPOOLS. Hence, despite the advantages of CRISPR/Cas9 to modulate expression bidirectionally and in cis, approaches based on ASOs or siPOOLS may be the better choice to target specifically the transcript from complex loci.

Gray, E. E., D. Winship, et al. "The AIM2-like Receptors Are Dispensable for the Interferon Response to Intracellular DNA." *Immunity*. 2016 Aug 16;45(2):255-66. doi: 10.1016/j.immuni.2016.06.015. Epub 2016 Aug 2.

Detection of intracellular DNA triggers activation of the STING-dependent interferon-stimulatory DNA (ISD) pathway, which is essential for antiviral responses. Multiple DNA sensors have been proposed to activate this pathway, including AIM2-like receptors (ALRs). Whether the ALRs are essential for activation of this pathway remains unknown. To rigorously explore the function of ALRs, we generated mice lacking all 13 ALR genes. We found that ALRs are dispensable for the type I interferon (IFN) response to transfected DNA ligands, DNA virus infection, and lentivirus infection. We also found that ALRs do not contribute to autoimmune disease in the *Trex1*(-/-) mouse model of Aicardi-Goutieres Syndrome. Finally, CRISPR-mediated disruption of the human AIM2-like receptor IFI16 in primary fibroblasts revealed that IFI16 is not essential for the IFN response to human cytomegalovirus infection. Our findings indicate that ALRs are dispensable for the ISD response and suggest that alternative functions for these receptors should be explored.

Gwiazda, K. S., A. E. Grier, et al. "High Efficiency CRISPR/Cas9-mediated Gene Editing in Primary Human T-cells Using Mutant Adenoviral E4orf6/E1b55k "Helper" Proteins." *Mol Ther*. 2016 Sep 29;24(9):1570-80. doi: 10.1038/mt.2016.105. Epub 2016 May 16.

Many future therapeutic applications of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 and related RNA-guided nucleases are likely to require their use to promote gene targeting, thus necessitating development of methods that provide for delivery of three components-Cas9, guide RNAs and recombination templates-to primary cells rendered proficient for homology-directed repair. Here, we demonstrate an electroporation/transduction codelivery method that

utilizes mRNA to express both Cas9 and mutant adenoviral E4orf6 and E1b55k helper proteins in association with adeno-associated virus (AAV) vectors expressing guide RNAs and recombination templates. By transiently enhancing target cell permissiveness to AAV transduction and gene editing efficiency, this novel approach promotes efficient gene disruption and/or gene targeting at multiple loci in primary human T-cells, illustrating its broad potential for application in translational gene editing.

Ha, N. H., J. Long, et al. "The Circadian Rhythm Gene *Arntl2* Is a Metastasis Susceptibility Gene for Estrogen Receptor-Negative Breast Cancer." *PLoS Genet*. 2016 Sep 22;12(9):e1006267. doi: 10.1371/journal.pgen.1006267. eCollection 2016 Sep.

Breast cancer mortality is primarily due to metastasis rather than primary tumors, yet relatively little is understood regarding the etiology of metastatic breast cancer. Previously, using a mouse genetics approach, we demonstrated that inherited germline polymorphisms contribute to metastatic disease, and that these single nucleotide polymorphisms (SNPs) could be used to predict outcome in breast cancer patients. In this study, a backcross between a highly metastatic (FVB/NJ) and low metastatic (MOLF/EiJ) mouse strain identified *Arntl2*, a gene encoding a circadian rhythm transcription factor, as a metastasis susceptibility gene associated with progression, specifically in estrogen receptor-negative breast cancer patients. Integrated whole genome sequence analysis with DNase hypersensitivity sites reveals SNPs in the predicted promoter of *Arntl2*. Using CRISPR/Cas9-mediated substitution of the MOLF promoter, we demonstrate that the SNPs regulate *Arntl2* transcription and affect metastatic burden. Finally, analysis of SNPs associated with *ARNTL2* expression in human breast cancer patients revealed reproducible associations of *ARNTL2* expression quantitative trait loci (eQTL) SNPs with disease-free survival, consistent with the mouse studies.

Haas, S. A., V. Dettmer, et al. "Therapeutic genome editing with engineered nucleases." *Hamostaseologie*. 2017 Jan 10. doi: 10.5482/HAMO-16-09-0035.

Targeted genome editing with designer nucleases, such as zinc finger nucleases, TALE nucleases, and CRISPR-Cas nucleases, has heralded a new era in gene therapy. Genetic disorders, which have not been amenable to conventional gene-addition-type gene therapy approaches, such as disorders with dominant inheritance or diseases caused by mutations in tightly regulated genes, can now be treated by precise genome surgery. Moreover, engineered nucleases enable novel genetic interventions to fight infectious diseases or to improve

cancer immunotherapies. Here, we review the development of the different classes of programmable nucleases, discuss the challenges and improvements in translating gene editing into clinical use, and give an outlook on what applications can expect to enter the clinic in the near future.

Harada, T., H. Yamamoto, et al. "Wnt5b-associated exosomes promote cancer cell migration and proliferation." *Cancer Sci.* 2016 Oct 20. doi: [10.1111/cas.13109](https://doi.org/10.1111/cas.13109).

Wnt5b is a member of the same family of proteins as Wnt5a, the overexpression of which is associated with cancer aggressiveness. Wnt5b is also suggested to be involved in cancer progression, however, details remain unclarified. We analyzed the biochemical properties of purified Wnt5b and the mode of secretion of Wnt5b by cancer cells. Wnt5b was glycosylated at three asparagine residues and lipidated at one serine residue, and these post-translational modifications of Wnt5b were essential for secretion. Purified Wnt5b showed Dvl2 phosphorylation and Rac activation abilities to a similar extent as Wnt5a. In cultured-cell conditioned medium, Wnt5b was detected in supernatant or precipitation fractions that were separated by centrifugation at 100 000 g. In PANC-1 pancreatic cancer cells, 55% of secreted endogenous Wnt5b was associated with exosomes. Exosomes from wild-type PANC-1 cells, but not those from Wnt5b-knockout PANC-1 cells, activated Wnt5b signaling in CHO cells and stimulated migration and proliferation of A549 lung adenocarcinoma cells, suggesting that endogenous, Wnt5b-associated exosomes are active. The exosomes were taken up by CHO cells and immunoelectron microscopy revealed that Wnt5b is indeed associated with exosomes. In Caco-2 colon cancer cells, most Wnt5b was recovered in precipitation fractions when Wnt5b was ectopically expressed (Caco-2/Wnt5b cells). Knockdown of TSG101, an exosome marker, decreased the secretion of Wnt5b-associated exosomes from Caco-2/Wnt5b cells and inhibited Wnt5b-dependent cell proliferation. Exosomes secreted from Caco-2/Wnt5b cells stimulated migration and proliferation of A549 cells. These results suggest that Wnt5b-associated exosomes promote cancer cell migration and proliferation in a paracrine manner.

Harrod, A., J. Fulton, et al. "Genomic modelling of the ESR1 Y537S mutation for evaluating function and new therapeutic approaches for metastatic breast cancer." *Oncogene.* 2016 Oct 17. doi: [10.1038/onc.2016.382](https://doi.org/10.1038/onc.2016.382).

Drugs that inhibit estrogen receptor-alpha (ER) activity have been highly successful in treating

and reducing breast cancer progression in ER-positive disease. However, resistance to these therapies presents a major clinical problem. Recent genetic studies have shown that mutations in the ER gene are found in >20% of tumours that progress on endocrine therapies. Remarkably, the great majority of these mutations localize to just a few amino acids within or near the critical helix 12 region of the ER hormone binding domain, where they are likely to be single allele mutations. Understanding how these mutations impact on ER function is a prerequisite for identifying methods to treat breast cancer patients featuring such mutations. Towards this end, we used CRISPR-Cas9 genome editing to make a single allele knock-in of the most commonly mutated amino acid residue, tyrosine 537, in the estrogen-responsive MCF7 breast cancer cell line. Genomic analyses using RNA-seq and ER ChIP-seq demonstrated that the Y537S mutation promotes constitutive ER activity globally, resulting in estrogen-independent growth. MCF7-Y537S cells were resistant to the anti-estrogen tamoxifen and fulvestrant. Further, we show that the basal transcription factor TFIID is constitutively recruited by ER-Y537S, resulting in ligand-independent phosphorylation of Serine 118 (Ser118) by the TFIID kinase, cyclin-dependent kinase (CDK)7. The CDK7 inhibitor, THZ1 prevented Ser118 phosphorylation and inhibited growth of MCF7-Y537S cells. These studies confirm the functional importance of ER mutations in endocrine resistance, demonstrate the utility of knock-in mutational models for investigating alternative therapeutic approaches and highlight CDK7 inhibition as a potential therapy for endocrine-resistant breast cancer mediated by ER mutations. *Oncogene advance online publication*, 17 October 2016; doi:10.1038/onc.2016.382.

He, Y., M. Ye, et al. "High Rab11-FIP4 expression predicts poor prognosis and exhibits tumor promotion in pancreatic cancer." *Int J Oncol.* 2016 Dec 30. doi: [10.3892/ijco.2016.3828](https://doi.org/10.3892/ijco.2016.3828).

Some studies have demonstrated that Rab11-family interacting proteins (Rab11-FIPs) are connected with the tumorigenesis, and they may act as tumor promoters in some cancers. The clinicopathological significance of Rab11-family interacting protein 4 (Rab11-FIP4) expression and its possible effects on pancreatic cancer (PC) are still undiscovered. In this study, Rab11-FIP4 protein expression level in 60 PC specimens and pair-matched non-cancerous samples were detected by immunohistochemistry analysis. The results were analysed and compared with each patients' clinical data. Rab11-FIP4 expression in PC tissues increased significantly more than that of adjacent non-cancerous tissues (P=0.0001). Overexpression of Rab11-FIP4 in the PC tissues was

significantly related to tumor size ($P=0.0001$), histological grade ($P=0.028$), metastasis ($P=0.001$) and TNM stage ($P=0.004$) but not with age ($P=0.832$), gender ($P=0.228$) or tumor site ($P=0.875$). Kaplan-Meier survival analysis showed that overexpression of Rab11-FIP4 was significantly related to overall survival time ($P=0.0036$). In addition, Rab11-FIP4 in PANC-1 pancreatic cancer cells were successfully knocked-out using the CRISPR/Cas9 system. Rab11-FIP4 knockout in PANC-1 cells inhibited cell growth, invasion and metastasis, and arrested cell cycle progression, but did not alter apoptosis. Our findings suggest that overexpression of Rab11-FIP4 predicts poor clinical outcomes for pancreatic cancer and contributes to pancreatic tumor progression.

Herold, N., S. G. Rudd, et al. "Targeting SAMHD1 with the Vpx protein to improve cytarabine therapy for hematological malignancies." *Nat Med.* 2017 Jan 9. doi: [10.1038/nm.4265](https://doi.org/10.1038/nm.4265).

The cytostatic deoxycytidine analog cytarabine (ara-C) is the most active agent available against acute myelogenous leukemia (AML). Together with anthracyclines, ara-C forms the backbone of AML treatment for children and adults. In AML, both the cytotoxicity of ara-C in vitro and the clinical response to ara-C therapy are correlated with the ability of AML blasts to accumulate the active metabolite ara-C triphosphate (ara-CTP), which causes DNA damage through perturbation of DNA synthesis. Differences in expression levels of known transporters or metabolic enzymes relevant to ara-C only partially account for patient-specific differential ara-CTP accumulation in AML blasts and response to ara-C treatment. Here we demonstrate that the deoxynucleoside triphosphate (dNTP) triphosphohydrolase SAM domain and HD domain 1 (SAMHD1) promotes the detoxification of intracellular ara-CTP pools. Recombinant SAMHD1 exhibited ara-CTPase activity in vitro, and cells in which SAMHD1 expression was transiently reduced by treatment with the simian immunodeficiency virus (SIV) protein Vpx were dramatically more sensitive to ara-C-induced cytotoxicity. CRISPR-Cas9-mediated disruption of the gene encoding SAMHD1 sensitized cells to ara-C, and this sensitivity could be abrogated by ectopic expression of wild-type (WT), but not dNTPase-deficient, SAMHD1. Mouse models of AML lacking SAMHD1 were hypersensitive to ara-C, and treatment ex vivo with Vpx sensitized primary patient-derived AML blasts to ara-C. Finally, we identified SAMHD1 as a risk factor in cohorts of both pediatric and adult patients with de novo AML who received ara-C treatment. Thus, SAMHD1 expression levels dictate patient sensitivity to ara-C, providing proof-of-concept that the targeting of SAMHD1 by Vpx could

be an attractive therapeutic strategy for potentiating ara-C efficacy in hematological malignancies.

Hesler, R. A., J. J. Huang, et al. "TGF-beta-induced stromal CYR61 promotes resistance to gemcitabine in pancreatic ductal adenocarcinoma through downregulation of the nucleoside transporters hENT1 and hCNT3." *Carcinogenesis.* 2016 Sep 7. pii: [bgw093](https://doi.org/10.1093/carcin/bgw093).

Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer in part due to inherent resistance to chemotherapy, including the first-line drug gemcitabine. Although low expression of the nucleoside transporters hENT1 and hCNT3 that mediate cellular uptake of gemcitabine has been linked to gemcitabine resistance, the mechanisms regulating their expression in the PDAC tumor microenvironment are largely unknown. Here, we report that the matricellular protein cysteine-rich angiogenic inducer 61 (CYR61) negatively regulates the nucleoside transporters hENT1 and hCNT3. CRISPR/Cas9-mediated knockout of CYR61 increased expression of hENT1 and hCNT3, increased cellular uptake of gemcitabine and sensitized PDAC cells to gemcitabine-induced apoptosis. In PDAC patient samples, expression of hENT1 and hCNT3 negatively correlates with expression of CYR61. We demonstrate that stromal pancreatic stellate cells (PSCs) are a source of CYR61 within the PDAC tumor microenvironment. Transforming growth factor-beta (TGF-beta) induces the expression of CYR61 in PSCs through canonical TGF-beta-ALK5-Smad2/3 signaling. Activation of TGF-beta signaling or expression of CYR61 in PSCs promotes resistance to gemcitabine in PDAC cells in an in vitro co-culture assay. Our results identify CYR61 as a TGF-beta-induced stromal-derived factor that regulates gemcitabine sensitivity in PDAC and suggest that targeting CYR61 may improve chemotherapy response in PDAC patients.

Hess, G. T., L. Fresard, et al. "Directed evolution using dCas9-targeted somatic hypermutation in mammalian cells." *Nat Methods.* 2016 Dec;13(12):1036-1042. doi: [10.1038/nmeth.4038](https://doi.org/10.1038/nmeth.4038). Epub 2016 Oct 31.

Engineering and study of protein function by directed evolution has been limited by the technical requirement to use global mutagenesis or introduce DNA libraries. Here, we develop CRISPR-X, a strategy to repurpose the somatic hypermutation machinery for protein engineering in situ. Using catalytically inactive dCas9 to recruit variants of cytidine deaminase (AID) with MS2-modified sgRNAs, we can specifically mutagenize endogenous targets with limited off-target damage. This generates diverse libraries of localized point mutations and can target multiple genomic locations simultaneously. We mutagenize GFP and select for spectrum-shifted

variants, including EGFP. Additionally, we mutate the target of the cancer therapeutic bortezomib, PSMB5, and identify known and novel mutations that confer bortezomib resistance. Finally, using a hyperactive AID variant, we mutagenize loci both upstream and downstream of transcriptional start sites. These experiments illustrate a powerful approach to create complex libraries of genetic variants in native context, which is broadly applicable to investigate and improve protein function.

Hosain, S. B., S. K. Khiste, et al. "Inhibition of glucosylceramide synthase eliminates the oncogenic function of p53 R273H mutant in the epithelial-mesenchymal transition and induced pluripotency of colon cancer cells." *Oncotarget*. 2016 Sep 13;7(37):60575-60592. doi: 10.18632/oncotarget.11169.

Missense mutation of tumor suppressor p53, which exhibits oncogenic gain-of-function (GOF), not only promotes tumor progression, but also diminishes therapeutic efficacies of cancer treatments. However, it remains unclear how a p53 missense mutant contributes to induced pluripotency of cancer stem cells (CSCs) in tumors exposed to chemotherapeutic agents. More importantly, it may be possible to abrogate the GOF by restoring wild-type p53 activity, thereby overcoming the deleterious effects resulting from heterotetramer formation, which often compromises the efficacies of current approaches being used to reactivate p53 function. Herewith, we report that p53 R273H missense mutant urges cancer cells to spawn CSCs. SW48/TP53 cells, which heterozygously carry the p53 R273H hot-spot mutant (R273H/+, introduced by a CRISPR/Casp9 system), were subchronically exposed to doxorubicin in cell culture and in tumor-bearing mice. We found that p53-R273H (TP53-Dox) cells were drug-resistant and exhibited epithelial-mesenchymal transition (EMT) and increased numbers of CSCs (CD44v6+/CD133+), which resulted in enhanced wound healing and tumor formation. Inhibition of glucosylceramide synthase with d-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) sensitized p53-R273H cancer cells and tumor xenografts to doxorubicin treatments. Intriguingly, PDMP treatments restored wild-type p53 expression in heterozygous R273H mutant cells and in tumors, decreasing CSCs and sensitizing cells and tumors to treatments. This study demonstrated that p53-R273H promotes EMT and induced pluripotency of CSCs in cancer cells exposed to doxorubicin, mainly through Zeb1 and beta-catenin transcription factors. Our results further indicate that restoration of p53 through inhibition of ceramide glycosylation might be an effective treatment approach for targeting cancers heterozygously

harboring TP53 missense mutations.

Hu, Y., G. Shi, et al. "Switch telomerase to ALT mechanism by inducing telomeric DNA damages and dysfunction of ATRX and DAXX." *Sci Rep*. 2016 Aug 31;6:32280. doi: 10.1038/srep32280.

Activation of telomerase or alternative lengthening of telomeres (ALT) is necessary for tumours to escape from dysfunctional telomere-mediated senescence. Anti-telomerase drugs might be effective in suppressing tumour growth in approximately 85-90% of telomerase-positive cancer cells. However, there are still chances for these cells to bypass drug treatment after switching to the ALT mechanism to maintain their telomere integrity. But the mechanism underlying this switch is unknown. In this study, we used telomerase-positive cancer cells (HTC75) to discover the mechanism of the telomerase-ALT switch by inducing telomere-specific DNA damage, alpha-thalassemia X-linked syndrome protein (ATRX) knockdown and deletion of death associated protein (DAXX). Surprisingly, two important ALT hallmarks in the ALT-like HTC75 cells were observed after treatments: ALT-associated promyelocytic leukaemia bodies (APBs) and extrachromosomal circular DNA of telomeric repeats. Moreover, knocking out hTERT by utilizing the CRISPR/Cas9 technique led to telomere elongation in a telomerase-independent manner in ALT-like HTC75 cells. In summary, this is the first report to show that inducing telomeric DNA damage, disrupting the ATRX/DAXX complex and inhibiting telomerase activity in telomerase-positive cancer cells lead to the ALT switch.

Huang, G., P. A. Osmulski, et al. "TGF-beta signal rewiring sustains epithelial-mesenchymal transition of circulating tumor cells in prostate cancer xenograft hosts." *Oncotarget*. 2016 Nov 22;7(47):77124-77137. doi: 10.18632/oncotarget.12808.

Activation of TGF-beta signaling is known to promote epithelial-mesenchymal transition (EMT) for the development of metastatic castration-resistant prostate cancer (mCRPC). To determine whether targeting TGF-beta signaling alone is sufficient to mitigate mCRPC, we used the CRISPR/Cas9 genome-editing approach to generate a dominant-negative mutation of the cognate receptor TGFBR2 that attenuated TGF-beta signaling in mCRPC cells. As a result, the delicate balance of oncogenic homeostasis is perturbed, profoundly uncoupling proliferative and metastatic potential of TGFBR2-edited tumor xenografts. This signaling disturbance triggered feedback rewiring by enhancing ERK signaling known to promote EMT-driven metastasis. Circulating tumor cells displaying upregulated EMT genes had elevated biophysical deformity and an increase in interactions

with chaperone macrophages for facilitating metastatic extravasation. Treatment with an ERK inhibitor resulted in decreased aggressive features of CRPC cells in vitro. Therefore, combined targeting of TGF-beta and its backup partner ERK represents an attractive strategy for treating mCRPC patients.

Huang, K., C. Yang, et al. "The CRISPR/Cas9 system targeting EGFR exon 17 abrogates NF-kappaB activation via epigenetic modulation of UBXN1 in EGFRwt/vIII glioma cells." *Cancer Lett.* 2016 Dec 18;388:269-280. doi: [10.1016/j.canlet.2016.12.011](https://doi.org/10.1016/j.canlet.2016.12.011).

Worldwide, glioblastoma (GBM) is the most lethal and frequent intracranial tumor. Despite decades of study, the overall survival of GBM patients remains unchanged. epidermal growth factor receptor (EGFR) amplification and gene mutation are thought to be negatively correlated with prognosis. In this study, we used proteomics to determine that UBXN1 is a negative downstream regulator of the EGFR mutation vIII (EGFRvIII). Via bioinformatics analysis, we found that UBXN1 is a factor that can improve glioma patients' overall survival time. We also determined that the down-regulation of UBXN1 is mediated by the upregulation of H3K27me3 in the presence of EGFRvIII. Because NF-kappaB can be negatively regulated by UBXN1, we believe that EGFRwt/vIII activates NF-kappaB by suppressing UBXN1 expression. Importantly, we used the latest genomic editing tool, CRISPR/Cas9, to knockout EGFRwt/vIII on exon 17 and further proved that UBXN1 is negatively regulated by EGFRwt/vIII. Furthermore, knockout of EGFR/EGFRvIII could benefit GBM in vitro and in vivo, indicating that CRISPR/Cas9 is a promising therapeutic strategy for both EGFR amplification and EGFR mutation-bearing patients.

Hultquist, J. F., K. Schumann, et al. "A Cas9 Ribonucleoprotein Platform for Functional Genetic Studies of HIV-Host Interactions in Primary Human T Cells." *Cell Rep.* 2016 Oct 25;17(5):1438-1452. doi: [10.1016/j.celrep.2016.09.080](https://doi.org/10.1016/j.celrep.2016.09.080).

New genetic tools are needed to understand the functional interactions between HIV and human host factors in primary cells. We recently developed a method to edit the genome of primary CD4+ T cells by electroporation of CRISPR/Cas9 ribonucleoproteins (RNPs). Here, we adapted this methodology to a high-throughput platform for the efficient, arrayed editing of candidate host factors. CXCR4 or CCR5 knockout cells generated with this method are resistant to HIV infection in a tropism-dependent manner, whereas knockout of LEDGF or TNPO3 results in a tropism-independent reduction in infection. CRISPR/Cas9 RNPs can furthermore edit multiple genes simultaneously, enabling studies of interactions among

multiple host and viral factors. Finally, in an arrayed screen of 45 genes associated with HIV integrase, we identified several candidate dependency/restriction factors, demonstrating the power of this approach as a discovery platform. This technology should accelerate target validation for pharmaceutical and cell-based therapies to cure HIV infection.

Hurwitz, S. N., D. Nkosi, et al. "CD63 regulates Epstein-Barr virus LMP1 exosomal packaging, enhancement of vesicle production, and non-canonical NF-kappaB signaling." *J Virol.* 2016 Dec 14. pii: [JVI.02251-16](https://doi.org/10.1128/JVI.02251-16).

Latent membrane protein 1 (LMP1) is an Epstein-Barr virus (EBV) encoded oncoprotein that is packaged into small extracellular vesicles (EVs) called exosomes. Trafficking of LMP1 into multivesicular bodies (MVBs) alters the content and function of exosomes. LMP1-modified exosomes enhance the growth, migration, and invasion of malignant cells, demonstrating the capacity to manipulate the tumor microenvironment and enhance the progression of EBV-associated cancers. Despite the growing evidence surrounding the significance of LMP1-modified exosomes in cancer, very little is understood about the mechanisms that orchestrate LMP1 incorporation into these vesicles. Recently, LMP1 was shown to co-purify with CD63, a conserved tetraspanin protein enriched in late endosomal and lysosomal compartments. Here, we demonstrate the importance of CD63 presence for exosomal packaging of LMP1. Nanoparticle tracking analysis and gradient purification revealed an increase in extracellular vesicle secretion and exosomal proteins following LMP1 expression. Immunoprecipitation of CD63-positive exosomes exhibited accumulation of LMP1 in this vesicle population. Functionally, CRISPR/Cas9 knockout of CD63 resulted in a reduction of LMP1-induced particle secretion. Furthermore, LMP1 packaging was severely impaired in CD63 knockout cells, concomitant with a disruption in the perinuclear localization of LMP1. Importantly, LMP1 trafficking to lipid rafts and activation of NF-kappaB and PI3K/AKT pathways remained intact following CD63 knockout, while MAPK/ERK and noncanonical NF-kappaB activation were observed to be increased. These results suggest that CD63 is a critical player in LMP1 exosomal trafficking, LMP1-mediated enhancement of exosome production, and may play further roles in limiting downstream LMP1 signaling. **IMPORTANCE:** EBV is a ubiquitous gamma herpesvirus linked to malignancies such as nasopharyngeal carcinoma, Burkitt's lymphoma, and Hodgkin's lymphoma. In the context of cancer, EBV hijacks the exosomal pathway to modulate cell-to-cell signaling by secreting viral components such as an

oncoprotein, LMP1 into host cell membrane-bound EVs. Trafficking of LMP1 into exosomes is associated with increased oncogenicity of these secreted vesicles. However, we have only a limited understanding of the mechanisms surrounding exosomal cargo packaging, including viral proteins. Here, we describe a role of LMP1 in EV production that requires CD63, and provide an extensive demonstration of CD63-mediated exosomal LMP1 release that is distinct from lipid raft trafficking. Finally, we present further evidence of the role of CD63 in limiting LMP1-induced non-canonical NF-kappaB and ERK activation. Our findings have implications in future investigations of physiological and pathological mechanisms of exosome biogenesis, protein trafficking, and signal transduction, especially in viral-associated tumorigenesis.

Jagle, S., A. Dertmann, et al. "ZEB1 is neither sufficient nor required for epithelial-mesenchymal transition in LS174T colorectal cancer cells." Biochem Biophys Res Commun. 2017 Jan 22;482(4):1226-1232. doi: 10.1016/j.bbrc.2016.12.017. Epub 2016 Dec 5.

Epithelial-mesenchymal transition (EMT) is implicated in metastases formation and acquired therapy resistance in several tumor entities. The two transcription factors SNAIL1 and ZEB1 are thought to be master regulators of EMT and to form a core regulatory network required for EMT-associated transcriptional reprogramming. Yet, inducible EMT models show the sequential upregulation first of SNAIL1 and only subsequently of ZEB1. Therefore, SNAIL1 and ZEB1 might be differentially needed for the onset and propagation of EMT. Here we used LS174T colorectal adenocarcinoma cells which do not express endogenous EMT-inducing transcription factors, to investigate whether ZEB1 is an obligatory downstream mediator of Snail1-induced EMT, and to test whether ZEB1 could elicit an EMT in a background of naive epithelial cells by itself. However, CRISPR/Cas9-mediated knockout of ZEB1 did not affect the ability of ectopically expressed Snail1 to trigger a complete EMT in ZEB1-deficient LS174T cells. In contrast to Snail1, ectopic ZEB1 had only minor effects on cell morphology and invasive growth in three-dimensional spheroid cultures. In agreement with this, expression of ZEB1 did not lead to repression of epithelial marker genes, and mesenchymal markers were not upregulated by ZEB1. Likewise, ectopic ZEB1 expression did not confer increased chemoresistance. We conclude that ZEB1 is neither required nor sufficient for EMT in LS174T colorectal cancer cells.

Jain, P. K., V. Ramanan, et al. "Development of Light-Activated CRISPR Using Guide RNAs with Photocleavable Protectors." Angew Chem Int Ed Engl.

2016 Sep 26;55(40):12440-4. doi: 10.1002/anie.201606123. Epub 2016 Aug 24.

The ability to remotely trigger CRISPR/Cas9 activity would enable new strategies to study cellular events with greater precision and complexity. In this work, we have developed a method to photocage the activity of the guide RNA called "CRISPR-plus" (CRISPR-precise light-mediated unveiling of sgRNAs). The photoactivation capability of our CRISPR-plus method is compatible with the simultaneous targeting of multiple DNA sequences and supports numerous modifications that can enable guide RNA labeling for use in imaging and mechanistic investigations.

Jerome, K. R. "Disruption or Excision of Provirus as an Approach to HIV Cure." AIDS Patient Care STDS. 2016 Dec;30(12):551-555. Epub 2016 Nov 17.

An effective approach to HIV cure will almost certainly require a combination of strategies, including some means of reducing the latent HIV reservoir. Because the integrated HIV provirus represents the major source of viral persistence and reactivation, one attractive approach is the direct targeting of provirus for disruption or excision using targeted endonucleases, such as CRISPR/Cas9, zinc finger nucleases, TAL effector nucleases, or meganucleases (homing endonucleases). This article highlights some of the challenges for successful endonuclease therapy for HIV, including optimization of enzyme activity and specificity, the possible emergence of viral resistance, and most importantly, efficient in vivo delivery of the enzymes to a sufficient portion of the latent reservoir.

Jin, H. J., S. Jung, et al. "Identification and validation of regulatory SNPs that modulate transcription factor chromatin binding and gene expression in prostate cancer." Oncotarget. 2016 Aug 23;7(34):54616-54626. doi: 10.18632/oncotarget.10520.

Prostate cancer (PCa) is the second most common solid tumor for cancer related deaths in American men. Genome wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with the increased risk of PCa. Because most of the susceptibility SNPs are located in noncoding regions, little is known about their functional mechanisms. We hypothesize that functional SNPs reside in cell type-specific regulatory elements that mediate the binding of critical transcription factors (TFs), which in turn result in changes in target gene expression. Using PCa-specific functional genomics data, here we identify 38 regulatory candidate SNPs and their target genes in PCa. Through risk analysis by incorporating gene expression and clinical data, we identify 6 target genes

(ZG16B, ANKRD5, RERE, FAM96B, NAALADL2 and GTPBP10) as significant predictors of PCa biochemical recurrence. In addition, 5 SNPs (rs2659051, rs10936845, rs9925556, rs6057110 and rs2742624) are selected for experimental validation using Chromatin immunoprecipitation (ChIP), dual-luciferase reporter assay in LNCaP cells, showing allele-specific enhancer activity. Furthermore, we delete the rs2742624-containing region using CRISPR/Cas9 genome editing and observe the drastic downregulation of its target gene UPK3A. Taken together, our results illustrate that this new methodology can be applied to identify regulatory SNPs and their target genes that likely impact PCa risk. We suggest that similar studies can be performed to characterize regulatory variants in other diseases.

Johnson, G. S., J. Li, et al. "A functional pseudogene, NMRAL2P, is regulated by Nrf2 and serves as a coactivator of NQO1 in sulforaphane-treated colon cancer cells." *Mol Nutr Food Res.* 2016 Nov 18. doi: [10.1002/mnfr.201600769](https://doi.org/10.1002/mnfr.201600769).

SCOPE: The anticancer agent sulforaphane (SFN) acts via multiple mechanisms to modulate gene expression, including the induction of nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-dependent signaling and the inhibition of histone deacetylase activity. Transcriptomics studies were performed in SFN-treated human colon cancer cells and in nontransformed colonic epithelial cells in order to pursue new mechanistic leads. METHODS AND RESULTS: RNA-sequencing corroborated the expected changes in cancer-related pathways after SFN treatment. In addition to NAD(P)H quinone dehydrogenase 1 (NQO1) and other well-known Nrf2-dependent targets, SFN strongly induced the expression of *Loc344887*. This noncoding RNA was confirmed as a novel functional pseudogene for NmrA-like redox sensor 1, and was given the name NmrA-like redox sensor 2 pseudogene (NMRAL2P). Chromatin immunoprecipitation experiments corroborated the presence of Nrf2 interactions on the NMRAL2P genomic region, and interestingly, NMRAL2P also served as a coregulator of NQO1 in human colon cancer cells. Silencing of NMRAL2P via CRISPR/Cas9 genome-editing protected against SFN-mediated inhibition of cancer cell growth, colony formation, and migration. CONCLUSION: NMRAL2P is the first functional pseudogene to be identified both as a direct transcriptional target of Nrf2, and as a downstream regulator of Nrf2-dependent NQO1 induction. Further studies are warranted on NMRAL2P-Nrf2 crosstalk and the associated mechanisms of gene regulation.

Kalhor, R., P. Mali, et al. "Rapidly evolving homing

CRISPR barcodes." *Nat Methods.* 2016 Dec 5. doi: [10.1038/nmeth.4108](https://doi.org/10.1038/nmeth.4108).

We present an approach for engineering evolving DNA barcodes in living cells. A homing guide RNA (hgRNA) scaffold directs the Cas9-hgRNA complex to the DNA locus of the hgRNA itself. We show that this homing CRISPR-Cas9 system acts as an expressed genetic barcode that diversifies its sequence and that the rate of diversification can be controlled in cultured cells. We further evaluate these barcodes in cell populations and show that they can be used to record lineage history and that the barcode RNA can be amplified in situ, a prerequisite for in situ sequencing. This integrated approach will have wide-ranging applications, such as in deep lineage tracing, cellular barcoding, molecular recording, dissecting cancer biology, and connectome mapping.

Kane, N. S., M. Vora, et al. "Efficient Screening of CRISPR/Cas9-Induced Events in Drosophila Using a Co-CRISPR Strategy." *G3 (Bethesda).* 2017 Jan 5;7(1):87-93. doi: [10.1534/g3.116.036723](https://doi.org/10.1534/g3.116.036723).

Genome editing using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and associated nuclease (Cas9) enables specific genetic modifications, including deletions, insertions, and substitutions in numerous organisms, such as the fruit fly *Drosophila melanogaster*. One challenge of the CRISPR/Cas9 system can be the laborious and time-consuming screening required to find CRISPR-induced modifications due to a lack of an obvious phenotype and low frequency after editing. Here we apply the successful co-CRISPR technique in *Drosophila* to simultaneously target a gene of interest and a marker gene, *ebony*, which is a recessive gene that produces dark body color and has the further advantage of not being a commonly used transgenic marker. We found that *Drosophila* broods containing higher numbers of CRISPR-induced *ebony* mutations ("jackpot" lines) are significantly enriched for indel events in a separate gene of interest, while broods with few or no *ebony* offspring showed few mutations in the gene of interest. Using two different PAM sites in our gene of interest, we report that approximately 61% (52-70%) of flies from the *ebony*-enriched broods had an indel in DNA near either PAM site. Furthermore, this marker mutation system may be useful in detecting the less frequent homology-directed repair events, all of which occurred in the *ebony*-enriched broods. By focusing on the broods with a significant number of *ebony* flies, successful identification of CRISPR-induced events is much faster and more efficient. The co-CRISPR technique we present significantly improves the screening efficiency in identification of genome-editing events in *Drosophila*.

Kang, J. T., B. Cho, et al. "Biallelic modification of IL2RG leads to severe combined immunodeficiency in pigs." Reprod Biol Endocrinol. 2016 Nov 3;14(1):74.

BACKGROUND: Pigs with SCID can be a useful model in regenerative medicine, xenotransplantation, and cancer cell transplantation studies. Utilizing genome editing technologies such as CRISPR/Cas9 system allows us to generate genetically engineered pigs at a higher efficiency. In this study, we report generation and phenotypic characterization of IL2RG knockout female pigs produced through combination of CRISPR/Cas9 system and SCNT. As expected, pigs lacking IL2RG presented SCID phenotype. **METHODS:** First, specific CRISPR/Cas9 systems targeting IL2RG were introduced into developing pig embryos then the embryos were transferred into surrogates. A total of six fetuses were obtained from the embryo transfer and fetal fibroblast cell lines were established. Then IL2RG knockout female cells carrying biallelic genetic modification were used as donor cells for SCNT, followed by embryo transfer. **RESULTS:** Three live cloned female piglets carrying biallelic mutations in IL2RG were produced. All cloned piglets completely lacked thymus and they had a significantly reduced level of mature T, B and NK cells in their blood and spleen. **CONCLUSIONS:** Here, we generated IL2RG knockout female pigs showing phenotypic characterization of SCID. This IL2RG knockout female pigs will be a promising model for biomedical and translational research.

Kang, J. T., J. Ryu, et al. "Generation of RUNX3 knockout pigs using CRISPR/Cas9-mediated gene targeting." Reprod Domest Anim. 2016 Dec;51(6):970-978. doi: 10.1111/rda.12775. Epub 2016 Oct 1.

Pigs are an attractive animal model to study the progression of cancer because of their anatomical and physiological similarities to human. However, the use of pig models for cancer research has been limited by availability of genetically engineered pigs which can recapitulate human cancer progression. Utilizing genome editing technologies such as CRISPR/Cas9 system allows us to generate genetically engineered pigs at a higher efficiency. In this study, specific CRISPR/Cas9 systems were used to target RUNX3, a known tumour suppressor gene, to generate a pig model that can induce gastric cancer in human. First, RUNX3 knockout cell lines carrying genetic modification (monoallelic or biallelic) of RUNX3 were generated by introducing engineered CRISPR/Cas9 system specific to RUNX3 into foetal fibroblast cells. Then, the genetically modified foetal fibroblast cells were used as donor cells for somatic cell nuclear transfer, followed by embryo transfer. We

successfully obtained four live RUNX3 knockout piglets from two surrogates. The piglets showed the lack of RUNX3 protein in their internal organ system. Our results demonstrate that the CRISPR/Cas9 system is effective in inducing mutations on a specific locus of genome and the RUNX3 knockout pigs can be useful resources for human cancer research and to develop novel cancer therapies.

Katigbak, A., R. Cencic, et al. "A CRISPR/Cas9 Functional Screen Identifies Rare Tumor Suppressors." Sci Rep. 2016 Dec 16;6:38968. doi: 10.1038/srep38968.

An enormous amount of tumor sequencing data has been generated through large scale sequencing efforts. The functional consequences of the majority of mutations identified by such projects remain an open, unexplored question. This problem is particularly complicated in the case of rare mutations where frequency of occurrence alone or prediction of functional consequences are insufficient to distinguish driver from passenger or bystander mutations. We combine genome editing technology with a powerful mouse cancer model to uncover previously unsuspected rare oncogenic mutations in Burkitt's lymphoma. We identify two candidate tumor suppressors whose loss cooperate with MYC over-expression to accelerate lymphomagenesis. Our results highlight the utility of in vivo CRISPR/Cas9 screens combined with powerful mouse models to identify and validate rare oncogenic modifier events from tumor mutational data.

Kehler, J., M. Greco, et al. "RNA-Generated and Gene-Edited Induced Pluripotent Stem Cells for Disease Modeling and Therapy." J Cell Physiol. 2016 Sep 15. doi: 10.1002/jcp.25597.

Cellular reprogramming by epigenomic remodeling of chromatin holds great promise in the field of human regenerative medicine. As an example, human-induced Pluripotent Stem Cells (iPSCs) obtained by reprogramming of patient somatic cells are sufficiently similar to embryonic stem cells (ESCs) and can generate all cell types of the human body. Clinical use of iPSCs is dependent on methods that do not utilize genome altering transgenic technologies that are potentially unsafe and ethically unacceptable. Transient delivery of exogenous RNA into cells provides a safer reprogramming system to transgenic approaches that rely on exogenous DNA or viral vectors. RNA reprogramming may prove to be more suitable for clinical applications and provide stable starting cell lines for gene-editing, isolation, and characterization of patient iPSC lines. The introduction and rapid evolution of CRISPR/Cas9 gene-editing systems has provided a readily accessible research tool

to perform functional human genetic experiments. Similar to RNA reprogramming, transient delivery of mRNA encoding Cas9 in combination with guide RNA sequences to target specific points in the genome eliminates the risk of potential integration of Cas9 plasmid constructs. We present optimized RNA-based laboratory procedure for making and editing iPSCs. In the near-term these two powerful technologies are being harnessed to dissect mechanisms of human development and disease in vitro, supporting both basic, and translational research. *J. Cell. Physiol.* 9999: 1-8, 2016. (c) 2016 Wiley Periodicals, Inc.

Khan, F. A., N. S. Pandupuspitasari, et al. "CRISPR/Cas9 therapeutics: a cure for cancer and other genetic diseases." *Oncotarget.* 2016 Aug 9;7(32):52541-52552. doi: [10.18632/oncotarget.9646](https://doi.org/10.18632/oncotarget.9646).

Cancer is caused by a series of alterations in genome and epigenome mostly resulting in activation of oncogenes or inactivation of cancer suppressor genes. Genetic engineering has become pivotal in the treatment of cancer and other genetic diseases, especially the formerly-niche use of clustered regularly interspaced short palindromic repeats (CRISPR) associated with Cas9. In defining its superior use, we have followed the recent advances that have been made in producing CRISPR/Cas9 as a therapy of choice. We also provide important genetic mutations where CRISPRs can be repurposed to create adaptive immunity to fight carcinomas and edit genetic mutations causing it. Meanwhile, challenges to CRISPR technology are also discussed with emphasis on ability of pathogens to evolve against CRISPRs. We follow the recent developments on the function of CRISPRs with different carriers which can efficiently deliver it to target cells; furthermore, analogous technologies are also discussed along CRISPRs, including zinc-finger nuclease (ZFN) and transcription activator-like effector nucleases (TALENs). Moreover, progress in clinical applications of CRISPR therapeutics is reviewed; in effect, patients can have lower morbidity and/or mortality from the therapeutic method with least possible side-effects.

Kiessling, M. K., S. Schuierer, et al. "Identification of oncogenic driver mutations by genome-wide CRISPR-Cas9 dropout screening." *BMC Genomics.* 2016 Sep 9;17(1):723. doi: [10.1186/s12864-016-3042-2](https://doi.org/10.1186/s12864-016-3042-2).

BACKGROUND: Genome-wide CRISPR-Cas9 dropout screens can identify genes whose knockout affects cell viability. Recent CRISPR screens detected thousands of essential genes required for cellular survival and key cellular processes; however discovering novel lineage-specific genetic dependencies from the many hits still remains a challenge. **RESULTS:** To assess whether CRISPR-

Cas9 dropout screens can help identify cancer dependencies, we screened two human cancer cell lines carrying known and distinct oncogenic mutations using a genome-wide sgRNA library. We found that the gRNA targeting the driver mutation EGFR was one of the highest-ranking candidates in the EGFR-mutant HCC-827 lung adenocarcinoma cell line. Likewise, sgRNAs for NRAS and MAP2K1 (MEK1), a downstream kinase of mutant NRAS, were identified among the top hits in the NRAS-mutant neuroblastoma cell line CHP-212. Depletion of these genes targeted by the sgRNAs strongly correlated with the sensitivity to specific kinase inhibitors of the EGFR or RAS pathway in cell viability assays. In addition, we describe other dependencies such as TBK1 in HCC-827 cells and TRIB2 in CHP-212 cells which merit further investigation. **CONCLUSIONS:** We show that genome-wide CRISPR dropout screens are suitable for the identification of oncogenic drivers and other essential genes.

Kim, E., C. Hurtz, et al. "Ibrutinib inhibits pre-BCR+ B-cell acute lymphoblastic leukemia progression by targeting BTK and BLK." *Blood.* 2016 Dec 28. pii: [blood-2016-06-722900](https://doi.org/10.1182/blood-2016-06-722900). doi: [10.1182/blood-2016-06-722900](https://doi.org/10.1182/blood-2016-06-722900).

Targeting B cell receptor (BCR) signaling is a successful therapeutic strategy in mature B cell malignancies. Precursor BCR (pre-BCR) signaling, which is critical during normal B lymphopoiesis, also plays an important role in pre-BCR+ B cell acute lymphoblastic leukemia (B-ALL). Here, we investigated the activity and mechanism of action of the BTK inhibitor ibrutinib in preclinical models of B-ALL. Pre-BCR+ ALL cells were exquisitely sensitive to ibrutinib at therapeutically relevant drug concentrations. In pre-BCR+ ALL, ibrutinib thwarted autonomous and induced pre-BCR signaling, resulting in deactivation of PI3K/AKT signaling. Ibrutinib modulated the expression of pre-BCR regulators (PTPN6, CD22, CD72 and PKC β) and substantially reduced BCL6 levels. Ibrutinib inhibited ALL cells migration towards CXCL12 and beneath marrow stromal cells, and reduced CD44 expression. CRISPR-Cas9 gene editing revealed that both, BTK and B lymphocyte kinase (BLK), are relevant targets of ibrutinib in pre-BCR+ ALL. Consequently, in mouse xenograft models of pre-BCR+ ALL ibrutinib treatment significantly prolonged survival. Combination treatment of ibrutinib with dexamethasone or vincristine demonstrated synergistic activity against pre-BCR+ ALL. These data corroborate ibrutinib as a promising targeted agent for pre-BCR+ ALL and highlight the importance of ibrutinib effects on alternative kinase targets.

Kim, E. J., K. H. Kang, et al. "CRISPR-Cas9: a promising tool for gene editing on induced pluripotent stem cells." Korean J Intern Med. 2017 Jan;32(1):42-61. doi: 10.3904/kjim.2016.198. Epub 2017 Jan 1.

Recent advances in genome editing with programmable nucleases have opened up new avenues for multiple applications, from basic research to clinical therapy. The ease of use of the technology-and particularly clustered regularly interspaced short palindromic repeats (CRISPR)-will allow us to improve our understanding of genomic variation in disease processes via cellular and animal models. Here, we highlight the progress made in correcting gene mutations in monogenic hereditary disorders and discuss various CRISPR-associated applications, such as cancer research, synthetic biology, and gene therapy using induced pluripotent stem cells. The challenges, ethical issues, and future prospects of CRISPR-based systems for human research are also discussed.

Koferle, A., K. Worf, et al. "CORALINA: a universal method for the generation of gRNA libraries for CRISPR-based screening." BMC Genomics. 2016 Nov 14;17(1):917.

BACKGROUND: The bacterial CRISPR system is fast becoming the most popular genetic and epigenetic engineering tool due to its universal applicability and adaptability. The desire to deploy CRISPR-based methods in a large variety of species and contexts has created an urgent need for the development of easy, time- and cost-effective methods enabling large-scale screening approaches. **RESULTS:** Here we describe CORALINA (comprehensive gRNA library generation through controlled nuclease activity), a method for the generation of comprehensive gRNA libraries for CRISPR-based screens. CORALINA gRNA libraries can be derived from any source of DNA without the need of complex oligonucleotide synthesis. We show the utility of CORALINA for human and mouse genomic DNA, its reproducibility in covering the most relevant genomic features including regulatory, coding and non-coding sequences and confirm the functionality of CORALINA generated gRNAs. **CONCLUSIONS:** The simplicity and cost-effectiveness make CORALINA suitable for any experimental system. The unprecedented sequence complexities obtainable with CORALINA libraries are a necessary pre-requisite for less biased large scale genomic and epigenomic screens.

Kuhn, M. W., E. Song, et al. "Targeting Chromatin Regulators Inhibits Leukemogenic Gene Expression in NPM1 Mutant Leukemia." Cancer Discov. 2016 Oct;6(10):1166-1181. Epub 2016 Aug 17.

Homeobox (HOX) proteins and the receptor tyrosine kinase FLT3 are frequently highly expressed

and mutated in acute myeloid leukemia (AML). Aberrant HOX expression is found in nearly all AMLs that harbor a mutation in the Nucleophosmin (NPM1) gene, and FLT3 is concomitantly mutated in approximately 60% of these cases. Little is known about how mutant NPM1 (NPM1mut) cells maintain aberrant gene expression. Here, we demonstrate that the histone modifiers MLL1 and DOT1L control HOX and FLT3 expression and differentiation in NPM1mut AML. Using a CRISPR/Cas9 genome editing domain screen, we show NPM1mut AML to be exceptionally dependent on the menin binding site in MLL1. Pharmacologic small-molecule inhibition of the menin-MLL1 protein interaction had profound antileukemic activity in human and murine models of NPM1mut AML. Combined pharmacologic inhibition of menin-MLL1 and DOT1L resulted in dramatic suppression of HOX and FLT3 expression, induction of differentiation, and superior activity against NPM1mut leukemia. **SIGNIFICANCE:** MLL1 and DOT1L are chromatin regulators that control HOX, MEIS1, and FLT3 expression and are therapeutic targets in NPM1mut AML. Combinatorial small-molecule inhibition has synergistic on-target activity and constitutes a novel therapeutic concept for this common AML subtype. *Cancer Discov*; 6(10): 1166-81. (c)2016 AACR. See related commentary by Hourigan and Aplan, p. 1087 This article is highlighted in the In This Issue feature, p. 1069.

Kurata, M., S. K. Rathe, et al. "Using genome-wide CRISPR library screening with library resistant DCK to find new sources of Ara-C drug resistance in AML." Sci Rep. 2016 Nov 3;6:36199. doi: 10.1038/srep36199.

Acute myeloid leukemia (AML) can display de novo or acquired resistance to cytosine arabinoside (Ara-C), a primary component of induction chemotherapy. To identify genes capable of independently imposing Ara-C resistance, we applied a genome-wide CRISPR library to human U937 cells and exposed to them to Ara-C. Interestingly, all drug resistant clones contained guide RNAs for DCK. To avoid DCK gene modification, gRNA resistant DCK cDNA was created by the introduction of silent mutations. The CRISPR screening was repeated using the gRNA resistant DCK, and loss of SLC29A was identified as also being capable of conveying Ara-C drug resistance. To determine if loss of Dck results in increased sensitivity to other drugs, we conducted a screen of 446 FDA approved drugs using two Dck-defective BXH-2 derived murine AML cell lines and their Ara-C sensitive parental lines. Both cell lines showed an increase in sensitivity to prednisolone. Guide RNA resistant cDNA rescue was a legitimate strategy and multiple DCK or SLC29A deficient human cell clones were established with one clone

becoming prednisolone sensitive. Dck-defective leukemic cells may become prednisolone sensitive indicating prednisolone may be an effective adjuvant therapy in some cases of DCK-negative AML.

Ladd, B., A. M. Mazzola, et al. "Effective combination therapies in preclinical endocrine resistant breast cancer models harboring ER mutations." *Oncotarget*. 2016 Aug 23;7(34):54120-54136. doi: [10.18632/oncotarget.10852](https://doi.org/10.18632/oncotarget.10852).

Although endocrine therapy is successfully used to treat patients with estrogen receptor (ER) positive breast cancer, a substantial proportion of this population will relapse. Several mechanisms of acquired resistance have been described including activation of the mTOR pathway, increased activity of CDK4 and activating mutations in ER. Using a patient derived xenograft model harboring a common activating ER ligand binding domain mutation (D538G), we evaluated several combinatorial strategies using the selective estrogen receptor degrader (SERD) fulvestrant in combination with chromatin modifying agents, and CDK4/6 and mTOR inhibitors. In this model, fulvestrant binds WT and MT ER, reduces ER protein levels, and downregulated ER target gene expression. Addition of JQ1 or vorinostat to fulvestrant resulted in tumor regression (41% and 22% regression, respectively) though no efficacy was seen when either agent was given alone. Interestingly, although the CDK4/6 inhibitor palbociclib and mTOR inhibitor everolimus were efficacious as monotherapies, long-term delayed tumor growth was only observed when co-administered with fulvestrant. This observation was consistent with a greater inhibition of compensatory signaling when palbociclib and everolimus were co-dosed with fulvestrant. The addition of fulvestrant to JQ1, vorinostat, everolimus and palbociclib also significantly reduced lung metastatic burden as compared to monotherapy. The combination potential of fulvestrant with palbociclib or everolimus were confirmed in an MCF7 CRISPR model harboring the Y537S ER activating mutation. Taken together, these data suggest that fulvestrant may have an important role in the treatment of ER positive breast cancer with acquired ER mutations.

Lekomtsev, S., S. Alijanni, et al. "Efficient generation and reversion of chromosomal translocations using CRISPR/Cas technology." *BMC Genomics*. 2016 Sep 17;17(1):739. doi: [10.1186/s12864-016-3084-5](https://doi.org/10.1186/s12864-016-3084-5).

BACKGROUND: Chromosomal translocations are a hallmark of cancer cells and give rise to fusion oncogenes. To gain insight into the mechanisms governing tumorigenesis, adequate model cell lines are required. **RESULTS:** We employ the versatile CRISPR/Cas system to engineer cell lines in

which chromosomal translocations are either generated de novo (CD74-ROS1) or existing translocations are reverted back to the original configuration (BCR-ABL1). To this end, we co-apply two guide RNAs to artificially generate two breakpoints and screen for spontaneous fusion events by PCR. **CONCLUSIONS:** The approach we use is efficient and delivers clones bearing translocations in a predictable fashion. Detailed analysis suggests that the clones display no additional undesired alterations, implying that the approach is robust and precise.

Li, C. G., M. F. Pu, et al. "MicroRNA-1304 suppresses human non-small cell lung cancer cell growth in vitro by targeting heme oxygenase-1." *Acta Pharmacol Sin*. 2017 Jan;38(1):110-119. doi: [10.1038/aps.2016.92](https://doi.org/10.1038/aps.2016.92). Epub 2016 Sep 19.

Previous studies have shown that microRNA-1304 (miR-1304) is dysregulated in certain types of cancers, including non-small cell lung cancer (NSCLC), and might be involved in tumor survival and/or growth. In this study we investigated the direct target of miR-1304 and its function in NSCLC in vitro. Human lung adenocarcinoma cell lines (A549 and NCI-H1975) were studied. The cell proliferation and survival were investigated via cell counting, MTT and colony-formation assays. Cell apoptosis and cell cycle were examined using annexin V-PE/7-AAD and PI staining assays, respectively. The dual-luciferase reporter assay was used to verify post-transcriptional regulation of heme oxygenase-1 (HO-1) by miR-1304. CRISPR/Cas9 was used to deplete endogenous miR-1304. Overexpression of MiR-1304 significantly decreased the number and viability of NSCLC cells and colony formation, and induced cell apoptosis and G0/G1 phase cell cycle arrest. HO-1 was demonstrated to be a direct target of miR-1304 in NSCLC cells. Restoration of HO-1 expression by hemin (20 μmol/L) abolished the inhibition of miR-1304 on cell growth and rescued miR-1304-induced apoptosis in A549 cells. Suppression of endogenous miR-1304 with anti-1304 significantly increased HO-1 expression and promoted cell growth and survival in A549 cells. In 17 human NSCLC tissue samples, miR-1304 expression was significantly decreased, while HO-1 expression was significantly increased as compared to normal lung tissues. MicroRNA-1304 is a tumor suppressor and HO-1 is its direct target in NSCLC. The results suggest the potential for miR-1304 as a therapeutic target for NSCLC.

Li, R., L. Fang, et al. "Type I CRISPR-Cas targets endogenous genes and regulates virulence to evade mammalian host immunity." *Cell Res*. 2016 Dec;26(12):1273-1287. doi: [10.1038/cr.2016.135](https://doi.org/10.1038/cr.2016.135). Epub 2016 Nov 18.

Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated (Cas) systems in bacteria and archaea provide adaptive immunity against invading foreign nucleic acids. Previous studies suggest that certain bacteria employ their Type II CRISPR-Cas systems to target their own genes, thus evading host immunity. However, whether other CRISPR-Cas systems have similar functions during bacterial invasion of host cells remains unknown. Here we identify a novel role for Type I CRISPR-Cas systems in evading host defenses in *Pseudomonas aeruginosa* strain UCBPP-PA14. The Type I CRISPR-Cas system of PA14 targets the mRNA of the bacterial quorum-sensing regulator LasR to dampen the recognition by toll-like receptor 4, thus diminishing the pro-inflammatory responses of the host in cell and mouse models. Mechanistically, this nuclease-mediated RNA degradation requires a "5'-GGN-3'" recognition motif in the target mRNA, and HD and DExD/H domains in Cas3 of the Type I CRISPR-Cas system. As LasR and Type I CRISPR-Cas systems are ubiquitously present in bacteria, our findings elucidate an important common mechanism underlying bacterial virulence.

Li, T., D. Liu, et al. "Par3L enhances colorectal cancer cell survival by inhibiting Lkb1/AMPK signaling pathway." *Biochem Biophys Res Commun.* 2017 Jan 22;482(4):1037-1041. doi: 10.1016/j.bbrc.2016.11.154. Epub 2016 Nov 28.

Partitioning defective 3-like protein (Par3L) is a recently identified cell polarity protein that plays an important role in mammary stem cell maintenance. Previously, we showed that high expression of Par3L is associated with poor survival in malignant colorectal cancer (CRC), but the underlying mechanism remained unknown. To this end, we established a Par3L knockout colorectal cancer cell line using the CRISPR/Cas system. Interestingly, reduced proliferation, enhanced cell death and caspase-3 activation were observed in Par3L knockout (KO) cells as compared with wildtype (WT) cells. Consistent with previous studies, we showed that Par3L interacts with a tumor suppressor protein liver kinase B1 (Lkb1). Moreover, Par3L depletion resulted in abnormal activation of Lkb1/AMPK signaling cascade. Knockdown of Lkb1 in these cells could significantly reduce AMPK activity and partially rescue cell death caused by Par3L knockdown. Furthermore, we showed that Par3L KO cells were more sensitive to chemotherapies and irradiation. Together, these results suggest that Par3L is essential for colorectal cancer cell survival by inhibiting Lkb1/AMPK signaling pathway, and is a putative therapeutic target for CRC.

Li, X., W. Chen, et al. "microRNA-137 promotes apoptosis in ovarian cancer cells via the regulation of XIAP." *Br J Cancer.* 2017 Jan 3;116(1):66-76. doi: 10.1038/bjc.2016.379. Epub 2016 Nov 22.

BACKGROUND: microRNAs (miRNAs) have regulatory roles in various cellular processes, including apoptosis. Recently, X-linked inhibitor of apoptosis protein (XIAP) has been reported to be dysregulated in epithelial ovarian cancer (EOC). However, the mechanism underlying this dysregulation is largely unknown. **METHODS:** Using bioinformatics and a literature analysis, a panel of miRNAs dysregulated in EOC was chosen for further experimental confirmation from hundreds of miRNAs that were predicted to interact with the XIAP 3'UTR. A dual-luciferase reporter assay was employed to detect the interaction by cellular co-transfection of an miRNA expression vector and a reporter vector with the XIAP 3'UTR fused to a Renilla luciferase reporter. DAPI and TUNEL approaches were used to further determine the effects of an miR-137 mimic and inhibitor on cisplatin-induced apoptosis in ovarian cancer cells. **RESULTS:** We identified eight miRNAs by screening a panel of dysregulated miRNAs that may target the XIAP 3'UTR. The strongest inhibitory miRNA, miR-137, suppressed the activity of a luciferase reporter gene fused with the XIAP 3'UTR and decreased the levels of XIAP protein in SKOV3 ovarian cancer cells. Furthermore, forced expression of miR-137 increased cisplatin-induced apoptosis, and the depressed expression of miR-137 decreased cisplatin-induced apoptosis in SKOV3 and primary EOC cells. Consistently, the disruption of miR-137 via CRISPR/Cas9 inhibited apoptosis and upregulated XIAP in A2780 cells. Furthermore, the effect of miR-137 on apoptosis could be rescued by XIAP in SKOV3 cells. In addition, miR-137 expression is inversely correlated with the level of XIAP protein in both ovarian cancer tissues and cell lines. **CONCLUSIONS:** Our data suggest that multiple miRNAs can regulate XIAP via its 3'UTR. miR-137 can sensitise ovarian cancer cells to cisplatin-induced apoptosis, providing new insight into overcoming drug resistance in ovarian cancer.

Li, X., R. Wu, et al. "The present and future of genome editing in cancer research." *Hum Genet.* 2016 Sep;135(9):1083-92. doi: 10.1007/s00439-016-1713-3. Epub 2016 Jul 18.

The widespread use of high-throughput genome sequencing methods is profoundly changing the way we understand, classify, and treat human cancers. To make sense of the deluge of sequencing data generated in the clinic, more effective and rapid assessments of the functional relevance of newly discovered cancer-associated mutations are urgently

needed. In this review, we discuss how genome editing technologies are responding to this major challenge. Largely focusing on CRISPR-based methods, we will highlight their potential to accelerate discovery, discuss their current limitations, and speculate about future applications.

Li, X. T., Y. Jun, et al. "tCRISPRi: tunable and reversible, one-step control of gene expression." *Sci Rep.* 2016 Dec 20;6:39076. doi: 10.1038/srep39076.

The ability to control the level of gene expression is a major quest in biology. A widely used approach employs deletion of a nonessential gene of interest (knockout), or multi-step recombineering to move a gene of interest under a repressible promoter (knockdown). However, these genetic methods are laborious, and limited for quantitative study. Here, we report a tunable CRISPR-cas system, "tCRISPRi", for precise and continuous titration of gene expression by more than 30-fold. Our tCRISPRi system employs various previous advancements into a single strain: (1) We constructed a new strain containing a tunable arabinose operon promoter PBAD to quantitatively control the expression of CRISPR-(d)Cas protein over two orders of magnitude in a plasmid-free system. (2) tCRISPRi is reversible, and gene expression is repressed under knockdown conditions. (3) tCRISPRi shows significantly less than 10% leaky expression. (4) Most important from a practical perspective, construction of tCRISPRi to target a new gene requires only one-step of oligo recombineering. Our results show that tCRISPRi, in combination with recombineering, provides a simple and easy-to-implement tool for gene expression control, and is ideally suited for construction of both individual strains and high-throughput tunable knockdown libraries.

Lin, C., H. Li, et al. "Increasing the Efficiency of CRISPR/Cas9-mediated Precise Genome Editing of HSV-1 Virus in Human Cells." *Sci Rep.* 2016 Oct 7;6:34531. doi: 10.1038/srep34531.

Genetically modified HSV-1 viruses serve as promising vectors for tumour therapy and vaccine development. The CRISPR/Cas9 system is one of the most powerful tools for precise gene editing of the genomes of organisms. However, whether the CRISPR/Cas9 system can precisely and efficiently make gene replacements in the genome of HSV-1 remains essentially unknown. Here, we reported CRISPR/Cas9-mediated editing of the HSV-1 genome in human cells, including the knockout and replacement of large genes. In established cells stably expressing CRISPR/Cas9, gRNA in coordination with Cas9 could direct a precise cleavage within a pre-defined target region, and foreign genes were

successfully used to replace the target gene seamlessly by HDR-mediated gene replacement. Introducing the NHEJ inhibitor SCR7 to the CRISPR/Cas9 system greatly facilitated HDR-mediated gene replacement in the HSV-1 genome. We provided the first genetic evidence that two copies of the ICP0 gene in different locations on the same HSV-1 genome could be simultaneously modified with high efficiency and with no off-target modifications. We also developed a revolutionized isolation platform for desired recombinant viruses using single-cell sorting. Together, our work provides a significantly improved method for targeted editing of DNA viruses, which will facilitate the development of anti-cancer oncolytic viruses and vaccines.

Lin, F., L. Dong, et al. "An Efficient Light-Inducible P53 Expression System for Inhibiting Proliferation of Bladder Cancer Cell." *Int J Biol Sci.* 2016 Oct 18;12(10):1273-1278. eCollection 2016.

Optogenetic gene expression systems enable spatial-temporal modulation of gene transcription and cell behavior. Although applications in biomedicine are emerging, the utility of optogenetic gene switches remains elusive in cancer research due to the relative low gene activation efficiency. Here, we present an optimized CRISPR-Cas9-based light-inducible gene expression device that controls gene transcription in a dose-dependent manner. To prove the potential utility of this device, P53 was tested as a functional target in the bladder cancer cell models. It was illustrated that the light-induced P53 inhibited proliferation of 5637 and UMUC-3 cell effectively. The "light-on" gene expression system may demonstrate a novel therapeutic strategy for bladder cancer intervention.

Liu, F., J. Huang, et al. "Drug Discovery via Human-Derived Stem Cell Organoids." *Front Pharmacol.* 2016 Sep 22;7:334. eCollection 2016.

Patient-derived cell lines and animal models have proven invaluable for the understanding of human intestinal diseases and for drug development although both inherently comprise disadvantages and caveats. Many genetically determined intestinal diseases occur in specific tissue microenvironments that are not adequately modeled by monolayer cell culture. Likewise, animal models incompletely recapitulate the complex pathologies of intestinal diseases of humans and fall short in predicting the effects of candidate drugs. Patient-derived stem cell organoids are new and effective models for the development of novel targeted therapies. With the use of intestinal organoids from patients with inherited diseases, the potency and toxicity of drug candidates can be evaluated better. Moreover, owing to the novel clustered regularly interspaced short palindromic

repeats/CRISPR-associated protein-9 genome-editing technologies, researchers can use organoids to precisely modulate human genetic status and identify pathogenesis-related genes of intestinal diseases. Therefore, here we discuss how patient-derived organoids should be grown and how advanced genome-editing tools may be applied to research on modeling of cancer and infectious diseases. We also highlight practical applications of organoids ranging from basic studies to drug screening and precision medicine.

Liu, J., Y. Zhou, et al. "CRISPR/Cas9 in zebrafish: an efficient combination for human genetic diseases modeling." *Hum Genet.* 2017 Jan;136(1):1-12. doi: [10.1007/s00439-016-1739-6](https://doi.org/10.1007/s00439-016-1739-6). Epub 2016 Nov 2.

The next-generation sequencing identifies a growing number of candidate genes associated with human genetic diseases, which inevitably requires efficient methods to validate the causal links between genotype and phenotype. Recently, zebrafish, with sufficiently high-throughput capabilities, has become a favored option to study human pathogenesis. In addition, CRISPR/Cas9-based approaches have radically reduced the efforts to introduce targeted genome engineering in various organisms. Here, we systemically review the basic considerations in the design of gene editing in zebrafish with CRISPR/Cas9, and explore the potential of the combination of these two to support efficient functional analysis of human genetic variants.

Liu, L., Y. W. Phua, et al. "Homo- and Heterotypic Association Regulates Signaling by the SgK269/PEAK1 and SgK223 Pseudokinases." *J Biol Chem.* 2016 Oct 7;291(41):21571-21583. Epub 2016 Aug 16.

SgK269/PEAK1 is a pseudokinase and scaffolding protein that plays a critical role in regulating growth factor receptor signal output and is implicated in the progression of several cancers, including those of the breast, colon, and pancreas. SgK269 is structurally related to SgK223, a human pseudokinase that also functions as a scaffold but recruits a distinct repertoire of signaling proteins compared with SgK269. Structural similarities between SgK269 and SgK223 include a predicted alpha-helical region (designated CH) immediately preceding the conserved C-terminal pseudokinase (PK) domain. Structure-function analyses of SgK269 in MCF-10A mammary epithelial cells demonstrated a critical role for the CH and PK regions in promoting cell migration and Stat3 activation. Characterization of the SgK269 "interactome" by mass spectrometry-based proteomics identified SgK223 as a novel binding partner, and association of SgK269 with

SgK223 in cells was dependent on the presence of the CH and PK domains of both pseudokinases. Homotypic association of SgK269 and SgK223 was also demonstrated and exhibited the same structural requirements. Further analysis using pulldowns and size-exclusion chromatography underscored the critical role of the CH region in SgK269/SgK223 association. Importantly, although SgK269 bridged SgK223 to Grb2, it was unable to activate Stat3 or efficiently enhance migration in SgK223 knock-out cells generated by CRISPR/Cas9. These results reveal previously unrecognized interplay between two oncogenic scaffolds and demonstrate a novel signaling mechanism for pseudokinases whereby homotypic and heterotypic association is used to assemble scaffolding complexes with distinct binding properties and hence qualitatively regulate signal output.

Liu, Y., Y. Zhan, et al. "Directing cellular information flow via CRISPR signal conductors." *Nat Methods.* 2016 Nov;13(11):938-944. doi: [10.1038/nmeth.3994](https://doi.org/10.1038/nmeth.3994). Epub 2016 Sep 5.

The complex phenotypes of eukaryotic cells are controlled by decision-making circuits and signaling pathways. A key obstacle to implementing artificial connections in signaling networks has been the lack of synthetic devices for efficient sensing, processing and control of biological signals. By extending sgRNAs to include modified riboswitches that recognize specific signals, we can create CRISPR-Cas9-based 'signal conductors' that regulate transcription of endogenous genes in response to external or internal signals of interest. These devices can be used to construct all the basic types of Boolean logic gates that perform logical signal operations in mammalian cells without needing the layering of multiple genetic circuits. They can also be used to rewire cellular signaling events by constructing synthetic links that couple different signaling pathways. Moreover, this approach can be applied to redirect oncogenic signal transduction by controlling simultaneous bidirectional (ON-OFF) gene transcriptions, thus enabling reprogramming of the fate of cancer cells.

Ma, J., J. Koster, et al. "CRISPR-DO for genome-wide CRISPR design and optimization." *Bioinformatics.* 2016 Nov 1;32(21):3336-3338. Epub 2016 Jul 10.

MOTIVATION: Despite the growing popularity in using CRISPR/Cas9 technology for genome editing and gene knockout, its performance still relies on well-designed single guide RNAs (sgRNA). In this study, we propose a web application for the Design and Optimization (CRISPR-DO) of guide sequences that target both coding and non-coding regions in spCas9 CRISPR system across

human, mouse, zebrafish, fly and worm genomes. CRISPR-DO uses a computational sequence model to predict sgRNA efficiency, and employs a specificity scoring function to evaluate the potential of off-target effect. It also provides information on functional conservation of target sequences, as well as the overlaps with exons, putative regulatory sequences and single-nucleotide polymorphisms (SNPs). The web application has a user-friendly genome-browser interface to facilitate the selection of the best target DNA sequences for experimental design. AVAILABILITY AND IMPLEMENTATION: CRISPR-DO is available at <http://cistrome.org/crispr/> CONTACT: qiliu@tongji.edu.cn or hanxu@jimmy.harvard.edu or xsliu@jimmy.harvard.edu Supplementary information: Supplementary data are available at Bioinformatics online.

Manier, S., J. T. Powers, et al. "The LIN28B/let-7 axis is a novel therapeutic pathway in multiple myeloma." *Leukemia*. 2016 Nov 11. doi: 10.1038/leu.2016.296.

MYC is a major oncogenic driver of multiple myeloma (MM) and yet almost no therapeutic agents exist that target MYC in MM. Here we report that the let-7 biogenesis inhibitor LIN28B correlates with MYC expression in MM and is associated with adverse outcome. We also demonstrate that the LIN28B/let-7 axis modulates the expression of MYC, itself a let-7 target. Further, perturbation of the axis regulates the proliferation of MM cells in vivo in a xenograft tumor model. RNA-sequencing and gene set enrichment analyses of CRISPR-engineered cells further suggest that the LIN28/let-7 axis regulates MYC and cell cycle pathways in MM. We provide proof of principle for therapeutic regulation of MYC through let-7 with an LNA-GapmeR (locked nucleic acid-GapmeR) containing a let-7b mimic in vivo, demonstrating that high levels of let-7 expression repress tumor growth by regulating MYC expression. These findings reveal a novel mechanism of therapeutic targeting of MYC through the LIN28B/let-7 axis in MM that may impact other MYC-dependent cancers as well. *Leukemia* advance online publication, 11 November 2016; doi:10.1038/leu.2016.296.

Mao, C., M. Livezey, et al. "Antiestrogen Resistant Cell Lines Expressing Estrogen Receptor alpha Mutations Upregulate the Unfolded Protein Response and are Killed by BHPI." *Sci Rep*. 2016 Oct 7;6:34753. doi: 10.1038/srep34753.

Outgrowth of metastases expressing ERalpha mutations Y537S and D538G is common after endocrine therapy for estrogen receptor alpha (ERalpha) positive breast cancer. The effect of replacing wild type ERalpha in breast cancer cells with

these mutations was unclear. We used the CRISPR-Cas9 genome editing system and homology directed repair to isolate and characterize 14 T47D cell lines in which ERalphaY537S or ERalphaD538G replace one or both wild-type ERalpha genes. In 2-dimensional, and in quantitative anchorage-independent 3-dimensional cell culture, ERalphaY537S and ERalphaD538G cells exhibited estrogen-independent growth. A progestin further increased their already substantial proliferation in micromolar 4-hydroxytamoxifen and fulvestrant/ICI 182,780 (ICI). Our recently described ERalpha biomodulator, BHPI, which hyperactivates the unfolded protein response (UPR), completely blocked proliferation. In ERalphaY537S and ERalphaD538G cells, estrogen-ERalpha target genes were constitutively active and partially antiestrogen resistant. The UPR marker sp-XBP1 was constitutively activated in ERalphaY537S cells and further induced by progesterone in both cell lines. UPR-regulated genes associated with tamoxifen resistance, including the oncogenic chaperone BiP/GRP78, were upregulated. ICI displayed a greater than 2 fold reduction in its ability to induce ERalphaY537S and ERalphaD538G degradation. Progestins, UPR activation and perhaps reduced ICI-stimulated ERalpha degradation likely contribute to antiestrogen resistance seen in ERalphaY537S and ERalphaD538G cells.

Marko, T. A., G. A. Shamsan, et al. "Slit-Robo GTPase-Activating Protein 2 as a metastasis suppressor in osteosarcoma." *Sci Rep*. 2016 Dec 14;6:39059. doi: 10.1038/srep39059.

Osteosarcoma is the most common primary bone tumor, with metastatic disease responsible for most treatment failure and patient death. A forward genetic screen utilizing Sleeping Beauty mutagenesis in mice previously identified potential genetic drivers of osteosarcoma metastasis, including Slit-Robo GTPase-Activating Protein 2 (Srgap2). This study evaluates the potential role of SRGAP2 in metastases-associated properties of osteosarcoma cell lines through Srgap2 knockout via the CRISPR/Cas9 nuclease system and conditional overexpression in the murine osteosarcoma cell lines K12 and K7M2. Proliferation, migration, and anchorage independent growth were evaluated. RNA sequencing and immunohistochemistry of human osteosarcoma tissue samples were used to further evaluate the potential role of the Slit-Robo pathway in osteosarcoma. The effects of Srgap2 expression modulation in the murine OS cell lines support the hypothesis that SRGAP2 may have a role as a suppressor of metastases in osteosarcoma. Additionally, SRGAP2 and other genes in the Slit-Robo pathway have altered transcript levels in a subset of mouse and human osteosarcoma, and

SRGAP2 protein expression is reduced or absent in a subset of primary tumor samples. SRGAP2 and other axon guidance proteins likely play a role in osteosarcoma metastasis, with loss of SRGAP2 potentially contributing to a more aggressive phenotype.

Matsumoto, A., A. Pasut, et al. "mTORC1 and muscle regeneration are regulated by the LINC00961-encoded SPAR polypeptide." *Nature*. 2017 Jan 12;541(7636):228-232. doi: 10.1038/nature21034. Epub 2016 Dec 26.

Although long non-coding RNAs (lncRNAs) are non-protein-coding transcripts by definition, recent studies have shown that a fraction of putative small open reading frames within lncRNAs are translated. However, the biological significance of these hidden polypeptides is still unclear. Here we identify and functionally characterize a novel polypeptide encoded by the lncRNA LINC00961. This polypeptide is conserved between human and mouse, is localized to the late endosome/lysosome and interacts with the lysosomal v-ATPase to negatively regulate mTORC1 activation. This regulation of mTORC1 is specific to activation of mTORC1 by amino acid stimulation, rather than by growth factors. Hence, we termed this polypeptide 'small regulatory polypeptide of amino acid response' (SPAR). We show that the SPAR-encoding lncRNA is highly expressed in a subset of tissues and use CRISPR/Cas9 engineering to develop a SPAR-polypeptide-specific knockout mouse while maintaining expression of the host lncRNA. We find that the SPAR-encoding lncRNA is downregulated in skeletal muscle upon acute injury, and using this in vivo model we establish that SPAR downregulation enables efficient activation of mTORC1 and promotes muscle regeneration. Our data provide a mechanism by which mTORC1 activation may be finely regulated in a tissue-specific manner in response to injury, and a paradigm by which lncRNAs encoding small polypeptides can modulate general biological pathways and processes to facilitate tissue-specific requirements, consistent with their restricted and highly regulated expression profile.

Mazor, Y., K. F. Sachsenmeier, et al. "Enhanced tumor-targeting selectivity by modulating bispecific antibody binding affinity and format valence." *Sci Rep*. 2017 Jan 9;7:40098. doi: 10.1038/srep40098.

Bispecific antibodies are considered attractive bio-therapeutic agents owing to their ability to target two distinct disease mediators. Cross-arm avidity targeting of antigen double-positive cancer cells over single-positive normal tissue is believed to enhance the therapeutic efficacy, restrict major escape mechanisms and increase tumor-targeting selectivity, leading to

reduced systemic toxicity and improved therapeutic index. However, the interplay of factors regulating target selectivity is not well understood and often overlooked when developing clinically relevant bispecific therapeutics. We show in vivo that dual targeting alone is not sufficient to endow selective tumor-targeting, and report the pivotal roles played by the affinity of the individual arms, overall avidity and format valence. Specifically, a series of monovalent and bivalent bispecific IgGs composed of the anti-HER2 trastuzumab moiety paired with affinity-modulated VH and VL regions of the anti-EGFR GA201 mAb were tested for selective targeting and eradication of double-positive human NCI-H358 non-small cell lung cancer target tumors over single-positive, non-target NCI-H358-HER2 CRISPR knock out tumors in nude mice bearing dual-flank tumor xenografts. Affinity-reduced monovalent bispecific variants, but not their bivalent bispecific counterparts, mediated a greater degree of tumor targeting selectivity, while the overall efficacy against the targeted tumor was not substantially affected.

Mendoza-Parra, M. A., V. Malysheva, et al. "Reconstructed cell fate-regulatory programs in stem cells reveal hierarchies and key factors of neurogenesis." *Genome Res*. 2016 Nov;26(11):1505-1519. Epub 2016 Sep 20.

Cell lineages, which shape the body architecture and specify cell functions, derive from the integration of a plethora of cell intrinsic and extrinsic signals. These signals trigger a multiplicity of decisions at several levels to modulate the activity of dynamic gene regulatory networks (GRNs), which ensure both general and cell-specific functions within a given lineage, thereby establishing cell fates. Significant knowledge about these events and the involved key drivers comes from homogeneous cell differentiation models. Even a single chemical trigger, such as the morphogen all-trans retinoic acid (RA), can induce the complex network of gene-regulatory decisions that matures a stem/precursor cell to a particular step within a given lineage. Here we have dissected the GRNs involved in the RA-induced neuronal or endodermal cell fate specification by integrating dynamic RXRA binding, chromatin accessibility, epigenetic promoter epigenetic status, and the transcriptional activity inferred from RNA polymerase II mapping and transcription profiling. Our data reveal how RA induces a network of transcription factors (TFs), which direct the temporal organization of cognate GRNs, thereby driving neuronal/endodermal cell fate specification. Modeling signal transduction propagation using the reconstructed GRNs indicated critical TFs for neuronal cell fate specification, which were confirmed by

CRISPR/Cas9-mediated genome editing. Overall, this study demonstrates that a systems view of cell fate specification combined with computational signal transduction models provides the necessary insight in cellular plasticity for cell fate engineering. The present integrated approach can be used to monitor the in vitro capacity of (engineered) cells/tissues to establish cell lineages for regenerative medicine.

Michels, H., R. I. Seinstra, et al. "Identification of an evolutionary conserved structural loop that is required for the enzymatic and biological function of tryptophan 2,3-dioxygenase." *Sci Rep.* 2016 Dec 20;6:39199. doi: 10.1038/srep39199.

The enzyme TDO (tryptophan 2,3-dioxygenase; TDO-2 in *Caenorhabditis elegans*) is a potential therapeutic target to cancer but is also thought to regulate proteotoxic events seen in the progression of neurodegenerative diseases. To better understand its function and develop specific compounds that target TDO we need to understand the structure of this molecule. In *C. elegans* we compared multiple different CRISPR/Cas9-induced *tdo-2* deletion mutants and identified a motif of three amino acids (PLD) that is required for the enzymatic conversion of tryptophan to N-formylkynurenine. Loss of TDO-2's enzymatic activity in PDL deletion mutants was accompanied by an increase in motility during aging and a prolonged lifespan, which is in line with the previously observed phenotypes induced by a knockdown of the full enzyme. Comparison of sequence structures suggests that blocking this motif might interfere with haem binding, which is essential for the enzyme's activity. The fact that these three residues are situated in an evolutionary conserved structural loop of the enzyme suggests that the findings can be translated to humans. The identification of this specific loop region in TDO-2-essential for its catalytic function-will aid in the design of novel inhibitors to treat diseases in which the TDO enzyme is overexpressed or hyperactive.

Miles, L. A., R. J. Garippa, et al. "Design, execution, and analysis of pooled in vitro CRISPR/Cas9 screens." *FEBS J.* 2016 Sep;283(17):3170-80. doi: 10.1111/febs.13770. Epub 2016 Jun 16.

The recently described clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technology has proven to be an exquisitely powerful and invaluable method of genetic manipulation and/or modification. As such, many researchers have realized the potential of using the CRISPR/Cas9 system as a novel screening method for the identification of important proteins in biological processes and have designed short guide RNA libraries for an in vitro screening. The seminal papers describing these

libraries offer valuable information regarding methods for generating the short guide RNA libraries, creating cell lines containing these libraries, and specific details regarding the screening workflow. However, certain considerations are often overlooked that may be important when planning and performing a screen, including which CRISPR library to use and how to best analyze the resulting screen data. In this review, we offer suggestions to answer some of these questions that are not covered as deeply in the papers describing the available CRISPR libraries for an in vitro screening.

Miller, J. B., S. Zhang, et al. "Non-Viral CRISPR/Cas Gene Editing In Vitro and In Vivo Enabled by Synthetic Nanoparticle Co-Delivery of Cas9 mRNA and sgRNA." *Angew Chem Int Ed Engl.* 2017 Jan 19;56(4):1059-1063. doi: 10.1002/anie.201610209. Epub 2016 Dec 16.

CRISPR/Cas is a revolutionary gene editing technology with wide-ranging utility. The safe, non-viral delivery of CRISPR/Cas components would greatly improve future therapeutic utility. We report the synthesis and development of zwitterionic amino lipids (ZALs) that are uniquely able to (co)deliver long RNAs including Cas9 mRNA and sgRNAs. ZAL nanoparticle (ZNP) delivery of low sgRNA doses (15 nm) reduces protein expression by >90 % in cells. In contrast to transient therapies (such as RNAi), we show that ZNP delivery of sgRNA enables permanent DNA editing with an indefinitely sustained 95 % decrease in protein expression. ZNP delivery of mRNA results in high protein expression at low doses in vitro (<600 pM) and in vivo (1 mg kg⁻¹). Intravenous co-delivery of Cas9 mRNA and sgLoxP induced expression of floxed tdTomato in the liver, kidneys, and lungs of engineered mice. ZNPs provide a chemical guide for rational design of long RNA carriers, and represent a promising step towards improving the safety and utility of gene editing.

Montero, J. J., I. Lopez de Silanes, et al. "Telomeric RNAs are essential to maintain telomeres." *Nat Commun.* 2016 Aug 17;7:12534. doi: 10.1038/ncomms12534.

Telomeres are transcribed generating long non-coding RNAs known as TERRA. Deciphering the role of TERRA has been one of the unsolved issues of telomere biology in the past decade. This has been, in part, due to lack of knowledge on the TERRA loci, thus preventing functional genetic studies. Here, we describe that long non-coding RNAs with TERRA features are transcribed from the human 20q and Xp subtelomeres. Deletion of the 20q locus by using the CRISPR-Cas9 technology causes a dramatic decrease in TERRA levels, while deletion of the Xp locus does

not result in decreased TERRA levels. Strikingly, 20q-TERRA ablation leads to dramatic loss of telomere sequences and the induction of a massive DNA damage response. These findings identify chromosome 20q as a main TERRA locus in human cells and represent the first demonstration in any organism of the essential role of TERRA in the maintenance of telomeres.

Moos, W. H., C. A. Pinkert, et al. "Epigenetic Treatment of Persistent Viral Infections." Drug Dev Res. 2016 Oct 20. doi: 10.1002/ddr.21366.

Preclinical Research Approximately 2,500 years ago, Hippocrates used the word herpes as a medical term to describe lesions that appeared to creep or crawl on the skin, advocating heat as a possible treatment. During the last 50 years, pharmaceutical research has made great strides, and therapeutic options have expanded to include small molecule antiviral agents, protease inhibitors, preventive vaccines for a handful of the papillomaviruses, and even cures for hepatitis C virus infections. However, effective treatments for persistent and recurrent viral infections, particularly the highly prevalent herpesviruses, continue to represent a significant unmet medical need, affecting the majority of the world's population. Exploring the population diversity of the human microbiome and the effects its compositional variances have on the immune system, health, and disease are the subjects of intense investigational research and study. Among the collection of viruses, bacteria, fungi, and single-cell eukaryotes that comprise the human microbiome, the virome has been grossly understudied relative to the influence it exerts on human pathophysiology, much as mitochondria have until recently failed to receive the attention they deserve, given their critical biomedical importance. Fortunately, cellular epigenetic machinery offers a wealth of druggable targets for therapeutic intervention in numerous disease indications, including those outlined above. With advances in synthetic biology, engineering our body's commensal microorganisms to seek out and destroy pathogenic species is clearly on the horizon. This is especially the case given recent breakthroughs in genetic manipulation with tools such as the CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated) gene-editing platforms. Tying these concepts together with our previous work on the microbiome and neurodegenerative and neuropsychiatric diseases, we suggest that, because mammalian cells respond to a viral infection by triggering a cascade of antiviral innate immune responses governed substantially by the cell's mitochondria, small molecule carnitinooids represent a new class of therapeutics with potential widespread

utility against many infectious insults. Drug Dev Res. 2016. (c) 2016 Wiley Periodicals, Inc.

Morgenstern, J., T. Fleming, et al. "Loss of glyoxalase 1 induces compensatory mechanism to achieve dicarbonyl detoxification in mammalian Schwann cells." J Biol Chem. 2016 Dec 12. pii: jbc.M116.760132.

The glyoxalase system is a highly specific enzyme system existing in all mammalian cells which is responsible for the detoxification of dicarbonyl species, primarily methylglyoxal (MG). It has been implicated to play an essential role in preventing the increased formation of advanced glycation endproducts under certain pathological conditions. We have established the first glyoxalase 1 knock-out model (GLO1^{-/-}) in mammalian Schwann cells using CRISPR/Cas9 technique to investigate compensatory mechanisms. Neither elevated concentrations of MG nor associated protein modifications were observed in GLO1^{-/-} cells. Alternative detoxification of MG in GLO1^{-/-} is achieved by increased catalytic efficiency of aldose reductase towards hemithioacetal (product of glutathione and MG), most likely caused by S-nitrosylation of aldose reductase. The hemithioacetal is mainly converted into lactaldehyde, which is paralleled by a loss of reduced glutathione. Inhibition of aldose reductase in GLO1^{-/-} cells is associated with an increased sensitivity against MG, elevated intracellular MG levels and associated modifications, as well as increased oxidative stress. Our data suggest that aldose reductase can compensate the loss of GLO1. This might be of clinical importance within the context of neuronal diseases caused by an impaired glyoxalase system and elevated levels of dicarbonyl species, such as MG.

Moyes, K. W., N. A. Lieberman, et al. "Genetically Engineered Macrophages: A Potential Platform for Cancer Immunotherapy." Hum Gene Ther. 2016 Oct 18.

In spite of their successes against hematologic malignancies, immunotherapeutic interventions for the treatment of patients with glioblastoma (GBM) have thus far been unsuccessful. This is in part due to the presence of a tumor microenvironment that fosters neoplastic growth and protects the tumor from destruction by the immune system. A novel genetically engineered macrophage-based platform has been developed with the potential to minimize the effects of the suppressive tumor microenvironment and improve innate and adaptive antitumor immune responses. A newly described lentiviral expression system was validated for the generation of transduced monocytes and monocyte-derived macrophages, and transgene expression was

shown to be stable over the course of weeks to months, both in vitro and in a mouse xenograft model of GBM. Furthermore, the genetically engineered macrophages (GEMs) neither caused morbidity in animals nor contributed to accelerated tumor growth. The versatility of GEMs is also highlighted by showing that they can be engineered to secrete proteins that either reduce immune suppression, such as the soluble transforming growth factor beta receptor II, or promote immune cell activation, by expressing interleukin 21. There is also the potential to prevent GEM-mediated immune suppression by using the CRISPR system to knock out genes responsible for dysfunction of cytotoxic cells, including interleukin 10 and programmed death-ligand 1. Together, these results suggest that GEMs are an ideal cell type for transforming the tumor microenvironment and enhancing antitumor immunity. Importantly, it is anticipated that these findings will have broad applicability to other types of tumors with microenvironments that currently preclude successful immunotherapeutic approaches.

Mulder, C. L., Y. Zheng, et al. "Spermatogonial stem cell autotransplantation and germline genomic editing: a future cure for spermatogenic failure and prevention of transmission of genomic diseases." Hum Reprod Update. 2016 Sep;22(5):561-73. doi: 10.1093/humupd/dmw017. Epub 2016 May 30.

BACKGROUND: Subfertility affects approximately 15% of all couples, and a severe male factor is identified in 17% of these couples. While the etiology of a severe male factor remains largely unknown, prior gonadotoxic treatment and genomic aberrations have been associated with this type of subfertility. Couples with a severe male factor can resort to ICSI, with either ejaculated spermatozoa (in case of oligozoospermia) or surgically retrieved testicular spermatozoa (in case of azoospermia) to generate their own biological children. Currently there is no direct treatment for azoospermia or oligozoospermia. Spermatogonial stem cell (SSC) autotransplantation (SSCT) is a promising novel clinical application currently under development to restore fertility in sterile childhood cancer survivors. Meanwhile, recent advances in genomic editing, especially the clustered regulatory interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9) system, are likely to enable genomic rectification of human SSCs in the near future. **OBJECTIVE AND RATIONALE:** The objective of this review is to provide insights into the prospects of the potential clinical application of SSCT with or without genomic editing to cure spermatogenic failure and to prevent transmission of genetic diseases. **SEARCH METHODS:** We performed a narrative

review using the literature available on PubMed not restricted to any publishing year on topics of subfertility, fertility treatments, (molecular regulation of) spermatogenesis and SSCT, inherited (genetic) disorders, prenatal screening methods, genomic editing and germline editing. For germline editing, we focussed on the novel CRISPR-Cas9 system. We included papers written in English only. **OUTCOMES:** Current techniques allow propagation of human SSCs in vitro, which is indispensable to successful transplantation. This technique is currently being developed in a preclinical setting for childhood cancer survivors who have stored a testis biopsy prior to cancer treatment. Similarly, SSCT could be used to restore fertility in sterile adult cancer survivors. In vitro propagation of SSCs might also be employed to enhance spermatogenesis in oligozoospermic men and in azoospermic men who still have functional SSCs albeit in insufficient numbers. The combination of SSCT with genomic editing techniques could potentially rectify defects in spermatogenesis caused by genomic mutations or, more broadly, prevent transmission of genomic diseases to the offspring. In spite of the promising prospects, SSCT and germline genomic editing are not yet clinically applicable and both techniques require optimization at various levels. **WIDER IMPLICATIONS:** SSCT with or without genomic editing could potentially be used to restore fertility in cancer survivors to treat couples with a severe male factor and to prevent the paternal transmission of diseases. This will potentially allow these couples to have their own biological children. Technical development is progressing rapidly, and ethical reflection and societal debate on the use of SSCT with or without genomic editing is pressing.

Naert, T., R. Colpaert, et al. "CRISPR/Cas9 mediated knockout of *rb1* and *rb1l* leads to rapid and penetrant retinoblastoma development in *Xenopus tropicalis*." Sci Rep. 2016 Oct 14;6:35264. doi: 10.1038/srep35264.

Retinoblastoma is a pediatric eye tumor in which bi-allelic inactivation of the Retinoblastoma 1 (RB1) gene is the initiating genetic lesion. Although recently curative rates of retinoblastoma have increased, there are at this time no molecular targeted therapies available. This is, in part, due to the lack of highly penetrant and rapid retinoblastoma animal models that facilitate rapid identification of targets that allow therapeutic intervention. Different mouse models are available, all based on genetic deactivation of both *Rb1* and Retinoblastoma-like 1 (*Rbl1*), and each showing different kinetics of retinoblastoma development. Here, we show by CRISPR/Cas9 techniques that similar to the mouse, neither *rb1* nor *rb1l* single mosaic mutant *Xenopus tropicalis* develop

tumors, whereas *rb1/rb1* double mosaic mutant tadpoles rapidly develop retinoblastoma. Moreover, occasionally presence of pinealoblastoma (trilateral retinoblastoma) was detected. We thus present the first CRISPR/Cas9 mediated cancer model in *Xenopus tropicalis* and the first genuine genetic non-mammalian retinoblastoma model. The rapid kinetics of our model paves the way for use as a pre-clinical model. Additionally, this retinoblastoma model provides unique possibilities for fast elucidation of novel drug targets by triple multiplex CRISPR/Cas9 gRNA injections (*rb1* + *rb1* + modifier gene) in order to address the clinically unmet need of targeted retinoblastoma therapy.

Narayanan, A., G. Hill-Teran, et al. "In vivo mutagenesis of miRNA gene families using a scalable multiplexed CRISPR/Cas9 nuclease system." *Sci Rep.* 2016 Aug 30;6:32386. doi: [10.1038/srep32386](https://doi.org/10.1038/srep32386).

A large number of microRNAs (miRNAs) are grouped into families derived from the same phylogenetic ancestors. miRNAs within a family often share the same physiological functions despite differences in their primary sequences, secondary structures, or chromosomal locations. Consequently, the generation of animal models to analyze the activity of miRNA families is extremely challenging. Using zebrafish as a model system, we successfully provide experimental evidence that a large number of miRNAs can be simultaneously mutated to abrogate the activity of an entire miRNA family. We show that injection of the Cas9 nuclease and two, four, ten, and up to twenty-four multiplexed single guide RNAs (sgRNAs) can induce mutations in 90% of the miRNA genomic sequences analyzed. We performed a survey of these 45 mutations in 10 miRNA genes, analyzing the impact of our mutagenesis strategy on the processing of each miRNA both computationally and in vivo. Our results offer an effective approach to mutate and study the activity of miRNA families and pave the way for further analysis on the function of complex miRNA families in higher multicellular organisms.

Neggers, J. E., E. Vanstreels, et al. "Heterozygous mutation of cysteine528 in XPO1 is sufficient for resistance to selective inhibitors of nuclear export." *Oncotarget.* 2016 Oct 18;7(42):68842-68850. doi: [10.18632/oncotarget.11995](https://doi.org/10.18632/oncotarget.11995).

Exportin-1 (CRM1/XPO1) is a crucial nuclear export protein that transports a wide variety of proteins from the nucleus to the cytoplasm. These cargo proteins include tumor suppressors and growth-regulatory factors and as such XPO1 is considered a potential anti-cancer target. From this perspective, inhibition of the XPO1-mediated nuclear export by selective inhibitor of nuclear export (SINE)

compounds has shown broad-spectrum anti-cancer activity. Furthermore, the clinical candidate SINE, selinexor, is currently in multiple phase I/II/IIb trials for treatment of cancer. Resistance against selinexor has not yet been observed in the clinic, but in vitro selection of resistance did not reveal any mutations in the target protein, XPO1. However, introduction of a homozygous mutation at the drug's target site, the cysteine 528 residue inside the XPO1 cargo-binding pocket, by genetic engineering, confers resistance to selinexor. Here we investigated whether this resistance to selinexor is recessive or dominant. For this purpose we have engineered multiple leukemia cell lines containing heterozygous or homozygous C528S substitutions using CRISPR/Cas9-mediated genome editing. Our findings show that heterozygous mutation confers similar resistance against selinexor as homozygous substitution, demonstrating that SINE resistance can be obtained by a single and dominant mutation of the cysteine528 residue in XPO1.

Nettersheim, D., S. Jostes, et al. "A signaling cascade including ARID1A, GADD45B and DUSP1 induces apoptosis and affects the cell cycle of germ cell cancers after romidepsin treatment." *Oncotarget.* 2016 Nov 15;7(46):74931-74946. doi: [10.18632/oncotarget.11647](https://doi.org/10.18632/oncotarget.11647).

In Western countries, the incidence of testicular germ cell cancers (GCC) is steadily rising over the last decades. Mostly, men between 20 and 40 years of age are affected. In general, patients suffering from GCCs are treated by orchietomy and radio- or chemotherapy. Due to resistance mechanisms, intolerance to the therapy or denial of chemo- / radiotherapy by the patients, GCCs are still a lethal threat, highlighting the need for alternative treatment strategies. In this study, we revealed that germ cell cancer cell lines are highly sensitive to the histone deacetylase inhibitor romidepsin in vitro and in vivo, highlighting romidepsin as a potential therapeutic option for GCC patients. Romidepsin-mediated inhibition of histone deacetylases led to disturbances of the chromatin landscape. This resulted in locus-specific histone-hyper- or hypoacetylation. We found that hypoacetylation at the ARID1A promoter caused repression of the SWI/SNF-complex member ARID1A. In consequence, this resulted in upregulation of the stress-sensors and apoptosis-regulators GADD45B, DUSP1 and CDKN1A. RNAi-driven knock down of ARID1A mimicked in parts the effects of romidepsin, while CRISPR/Cas9-mediated deletion of GADD45B attenuated the romidepsin-provoked induction of apoptosis and cell cycle alterations. We propose a signaling cascade involving ARID1A, GADD45B and DUSP1 as mediators of the romidepsin effects in GCC cells.

Niu, X., J. Zhao, et al. "Binding of Released Bim to Mcl-1 is a Mechanism of Intrinsic Resistance to ABT-199 which can be Overcome by Combination with Daunorubicin or Cytarabine in AML Cells." *Clin Cancer Res.* 2016 Sep 1;22(17):4440-51. doi: [10.1158/1078-0432.CCR-15-3057](https://doi.org/10.1158/1078-0432.CCR-15-3057). Epub 2016 Apr 21.

PURPOSE: To investigate the molecular mechanism underlying intrinsic resistance to ABT-199. **EXPERIMENTAL DESIGN:** Western blots and real-time RT-PCR were used to determine levels of Mcl-1 after ABT-199 treatment alone or in combination with cytarabine or daunorubicin. Immunoprecipitation of Bim and Mcl-1 were used to determine the effect of ABT-199 treatment on their interactions with Bcl-2 family members. Lentiviral short hairpin RNA knockdown of Bim and CRISPR knockdown of Mcl-1 were used to confirm their role in resistance to ABT-199. JC-1 assays and flow cytometry were used to determine drug-induced apoptosis. **RESULTS:** Immunoprecipitation of Bim from ABT-199-treated cell lines and a primary patient sample demonstrated decreased association with Bcl-2, but increased association with Mcl-1 without corresponding change in mitochondrial outer membrane potential. ABT-199 treatment resulted in increased levels of Mcl-1 protein, unchanged or decreased Mcl-1 transcript levels, and increased Mcl-1 protein half-life, suggesting that the association with Bim plays a role in stabilizing Mcl-1 protein. Combining conventional chemotherapeutic agent cytarabine or daunorubicin with ABT-199 resulted in increased DNA damage along with decreased Mcl-1 protein levels, compared with ABT-199 alone, and synergistic induction of cell death in both AML cell lines and primary patient samples obtained from AML patients at diagnosis. **CONCLUSIONS:** Our results demonstrate that sequestration of Bim by Mcl-1 is a mechanism of intrinsic ABT-199 resistance and supports the clinical development of ABT-199 in combination with cytarabine or daunorubicin for the treatment of AML. *Clin Cancer Res*; 22(17); 4440-51. (c)2016 AACR.

Noguchi, T., J. P. Ward, et al. "Temporally-Distinct PD-L1 Expression by Tumor and Host Cells Contributes to Immune Escape." *Cancer Immunol Res.* 2017 Jan 10. pii: [canimm.0391.2016](https://doi.org/10.1158/2326-6066.CIR-16-0391). doi: [10.1158/2326-6066.CIR-16-0391](https://doi.org/10.1158/2326-6066.CIR-16-0391).

Antibody blockade of Programmed Death-1 (PD-1) or its ligand, PD-L1, has led to unprecedented therapeutic responses in certain tumor-bearing individuals, but PD-L1 expression's prognostic value in stratifying cancer patients for such treatment remains unclear. Reports conflict on the significance of correlations between PD-L1 on tumor cells and positive clinical outcomes to PD-1/PD-L1 blockade.

We investigated this issue using genomically-related, clonal subsets from the same methylcholanthrene-induced sarcoma: a highly immunogenic subset that is spontaneously eliminated in vivo by adaptive immunity and a less immunogenic subset that forms tumors in immunocompetent mice, but is sensitive to PD-1/PD-L1 blockade therapy. Using CRISPR/Cas9-induced loss-of-function approaches and overexpression gain-of-function techniques, we confirmed that PD-L1 on tumor cells is key to promoting tumor escape. Additionally, the capacity of PD-L1 to suppresses antitumor responses was inversely proportional to tumor cell antigenicity. PD-L1 expression on host cells, particularly tumor-associated macrophages (TAMs), was also important for tumor immune escape. We demonstrated that induction of PD-L1 on tumor cells was interferon gamma (IFN γ)-dependent and transient, but PD-L1 induction on TAMs was of greater magnitude, only partially IFN γ dependent, and was stable over time. Thus, PD-L1 expression on either tumor cells or host immune cells could lead to tumor escape from immune control, indicating that total PD-L1 expression in the immediate tumor microenvironment may represent a more accurate biomarker for predicting response to PD-1/PD-L1 blockade therapy, compared to monitoring PD-L1 expression on tumor cells alone.

Oh, S., E. You, et al. "Genetic disruption of tubulin acetyltransferase, alphaTAT1, inhibits proliferation and invasion of colon cancer cells through decreases in Wnt1/beta-catenin signaling." *Biochem Biophys Res Commun.* 2017 Jan 1;482(1):8-14. doi: [10.1016/j.bbrc.2016.11.039](https://doi.org/10.1016/j.bbrc.2016.11.039). Epub 2016 Nov 9.

Microtubules are required for diverse cellular processes, and abnormal regulation of microtubule dynamics is closely associated with severe diseases including malignant tumors. In this study, we report that alpha-tubulin N-acetyltransferase (alphaTAT1), a regulator of alpha-tubulin acetylation, is required for colon cancer proliferation and invasion via regulation of Wnt1 and its downstream genes expression. Public transcriptome analysis showed that expression of ATAT1 is specifically upregulated in colon cancer tissue. A knockout (KO) of ATAT1 in the HCT116 colon cancer cell line, using the CRISPR/Cas9 system showed profound inhibition of proliferative and invasive activities of these cancer cells. Overexpression of alphaTAT1 or the acetyl-mimic K40Q alpha-tubulin mutant in alphaTAT1 KO cells restored the invasiveness, indicating that microtubule acetylation induced by alphaTAT1 is critical for HCT116 cell invasion. Analysis of colon cancer-related gene expression in alphaTAT1 KO cells revealed that the loss of alphaTAT1 decreased the

expression of WNT1. Mechanistically, abrogation of tubulin acetylation by alphaTAT1 knockout inhibited localization of beta-catenin to the plasma membrane and nucleus, thereby resulting in the downregulation of Wnt1 and of its downstream genes including CCND1, MMP-2, and MMP-9. These results suggest that alphaTAT1-mediated Wnt1 expression via microtubule acetylation is important for colon cancer progression.

Ou, Y., S. J. Wang, et al. "Activation of SAT1 engages polyamine metabolism with p53-mediated ferroptotic responses." Proc Natl Acad Sci U S A. 2016 Nov 1;113(44):E6806-E6812. Epub 2016 Oct 3.

Although p53-mediated cell-cycle arrest, senescence, and apoptosis remain critical barriers to cancer development, the emerging role of p53 in cell metabolism, oxidative responses, and ferroptotic cell death has been a topic of great interest. Nevertheless, it is unclear how p53 orchestrates its activities in multiple metabolic pathways into tumor suppressive effects. Here, we identified the SAT1 (spermidine/spermine N1-acetyltransferase 1) gene as a transcription target of p53. SAT1 is a rate-limiting enzyme in polyamine catabolism critically involved in the conversion of spermidine and spermine back to putrescine. Surprisingly, we found that activation of SAT1 expression induces lipid peroxidation and sensitizes cells to undergo ferroptosis upon reactive oxygen species (ROS)-induced stress, which also leads to suppression of tumor growth in xenograft tumor models. Notably, SAT1 expression is down-regulated in human tumors, and CRISPR-cas9-mediated knockout of SAT1 expression partially abrogates p53-mediated ferroptosis. Moreover, SAT1 induction is correlated with the expression levels of arachidonate 15-lipoxygenase (ALOX15), and SAT1-induced ferroptosis is significantly abrogated in the presence of PD146176, a specific inhibitor of ALOX15. Thus, our findings uncover a metabolic target of p53 involved in ferroptotic cell death and provide insight into the regulation of polyamine metabolism and ferroptosis-mediated tumor suppression.

Pan, Y., N. Shen, et al. "CRISPR RNA-guided FokI nucleases repair a PAH variant in a phenylketonuria model." Sci Rep. 2016 Oct 27;6:35794. doi: [10.1038/srep35794](https://doi.org/10.1038/srep35794).

The CRISPR/Cas9 system is a recently developed genome editing technique. In this study, we used a modified CRISPR system, which employs the fusion of inactive Cas9 (dCas9) and the FokI endonuclease (FokI-dCas9) to correct the most common variant (allele frequency 21.4%) in the phenylalanine hydroxylase (PAH) gene - c.1222C>T (p.Arg408Trp) - as an approach toward curing

phenylketonuria (PKU). PKU is the most common inherited diseases in amino acid metabolism. It leads to severe neurological and neuropsychological symptoms if untreated or late diagnosed. Correction of the disease-causing variants could rescue residual PAH activity and restore normal function. Co-expression of a single guide RNA plasmid, a FokI-dCas9-zsGreen1 plasmid, and the presence of a single-stranded oligodeoxynucleotide in PAH_c.1222C>T COS-7 cells - an in vitro model for PKU - corrected the PAH variant and restored PAH activity. Also in this system, the HDR enhancer RS-1 improved correction efficiency. This proof-of-concept indicates the potential of the FokI-dCas9 system for precision medicine, in particular for targeting PKU and other monogenic metabolic diseases.

Pankowicz, F. P., M. Barzi, et al. "Reprogramming metabolic pathways in vivo with CRISPR/Cas9 genome editing to treat hereditary tyrosinaemia." Nat Commun. 2016 Aug 30;7:12642. doi: [10.1038/ncomms12642](https://doi.org/10.1038/ncomms12642).

Many metabolic liver disorders are refractory to drug therapy and require orthotopic liver transplantation. Here we demonstrate a new strategy, which we call metabolic pathway reprogramming, to treat hereditary tyrosinaemia type I in mice; rather than edit the disease-causing gene, we delete a gene in a disease-associated pathway to render the phenotype benign. Using CRISPR/Cas9 in vivo, we convert hepatocytes from tyrosinaemia type I into the benign tyrosinaemia type III by deleting Hpd (hydroxyphenylpyruvate dioxygenase). Edited hepatocytes (Fah(-)/Hpd(-)) display a growth advantage over non-edited hepatocytes (Fah(-)/Hpd(+)) and, in some mice, almost completely replace them within 8 weeks. Hpd excision successfully reroutes tyrosine catabolism, leaving treated mice healthy and asymptomatic. Metabolic pathway reprogramming sidesteps potential difficulties associated with editing a critical disease-causing gene and can be explored as an option for treating other diseases.

Papagiannakopoulos, T., M. R. Bauer, et al. "Circadian Rhythm Disruption Promotes Lung Tumorigenesis." Cell Metab. 2016 Aug 9;24(2):324-31. doi: [10.1016/j.cmet.2016.07.001](https://doi.org/10.1016/j.cmet.2016.07.001). Epub 2016 Jul 28.

Circadian rhythms are 24-hr oscillations that control a variety of biological processes in living systems, including two hallmarks of cancer, cell division and metabolism. Circadian rhythm disruption by shift work is associated with greater risk for cancer development and poor prognosis, suggesting a putative tumor-suppressive role for circadian rhythm homeostasis. Using a genetically engineered mouse

model of lung adenocarcinoma, we have characterized the effects of circadian rhythm disruption on lung tumorigenesis. We demonstrate that both physiologic perturbation (jet lag) and genetic mutation of the central circadian clock components decreased survival and promoted lung tumor growth and progression. The core circadian genes *Per2* and *Bmal1* were shown to have cell-autonomous tumor-suppressive roles in transformation and lung tumor progression. Loss of the central clock components led to increased c-Myc expression, enhanced proliferation, and metabolic dysregulation. Our findings demonstrate that both systemic and somatic disruption of circadian rhythms contribute to cancer progression.

Park, J. S., J. H. Ryu, et al. "Innate Color Preference of Zebrafish and Its Use in Behavioral Analyses." *Mol Cells*. 2016 Oct;39(10):750-755. Epub 2016 Oct 31.

Although innate color preference of motile organisms may provide clues to behavioral biases, it has remained a longstanding question. In this study, we investigated innate color preference of zebrafish larvae. A cross maze with different color sleeves around each arm was used for the color preference test (R; red, G; green, B; blue, Y; yellow). The findings showed that 5 dpf zebrafish larvae preferred blue over other colors ($B > R > G > Y$). To study innate color recognition further, tyrosinase mutants were generated using CRISPR/Cas9 system. As a model for oculocutaneous albinism (OCA) and color vision impairment, tyrosinase mutants demonstrated diminished color sensation, indicated mainly by hypopigmentation of the retinal pigment epithelium (RPE). Due to its relative simplicity and ease, color preference screening using zebrafish larvae is suitable for high-throughput screening applications. This system may potentially be applied to the analysis of drug effects on larval behavior or the detection of sensory deficits in neurological disorder models, such as autism-related disorders, using mutant larvae generated by the CRISPR/Cas9 technique.

Park, R. J., T. Wang, et al. "A genome-wide CRISPR screen identifies a restricted set of HIV host dependency factors." *Nat Genet*. 2016 Dec 19. doi: [10.1038/ng.3741](https://doi.org/10.1038/ng.3741).

Host proteins are essential for HIV entry and replication and can be important nonviral therapeutic targets. Large-scale RNA interference (RNAi)-based screens have identified nearly a thousand candidate host factors, but there is little agreement among studies and few factors have been validated. Here we demonstrate that a genome-wide CRISPR-based screen identifies host factors in a physiologically relevant cell system. We identify five factors, including the HIV co-receptors CD4 and CCR5, that

are required for HIV infection yet are dispensable for cellular proliferation and viability. Tyrosylprotein sulfotransferase 2 (TPST2) and solute carrier family 35 member B2 (SLC35B2) function in a common pathway to sulfate CCR5 on extracellular tyrosine residues, facilitating CCR5 recognition by the HIV envelope. Activated leukocyte cell adhesion molecule (ALCAM) mediates cell aggregation, which is required for cell-to-cell HIV transmission. We validated these pathways in primary human CD4+ T cells through Cas9-mediated knockout and antibody blockade. Our findings indicate that HIV infection and replication rely on a limited set of host-dispensable genes and suggest that these pathways can be studied for therapeutic intervention.

Pattison, J. M., V. Posternak, et al. "Transcription Factor KLF5 Binds a Cyclin E1 Polymorphic Intronic Enhancer to Confer Increased Bladder Cancer Risk." *Mol Cancer Res*. 2016 Nov;14(11):1078-1086. Epub 2016 Aug 11.

It is well established that environmental toxins, such as exposure to arsenic, are risk factors in the development of urinary bladder cancer, yet recent genome-wide association studies (GWAS) provide compelling evidence that there is a strong genetic component associated with disease predisposition. A single-nucleotide polymorphism (SNP), rs8102137, was identified on chromosome 19q12, residing 6 kb upstream of the important cell-cycle regulator and proto-oncogene, Cyclin E1 (CCNE1). However, the functional role of this variant in bladder cancer predisposition has been unclear because it lies within a non-coding region of the genome. Here, it is demonstrated that bladder cancer cells heterozygous for this SNP exhibit biased allelic expression of CCNE1 with 1.5-fold more transcription occurring from the risk allele. Furthermore, using chromatin immunoprecipitation assays, a novel enhancer element was identified within the first intron of CCNE1 that binds Kruppel-like Factor 5 (KLF5), a known transcriptional activator in bladder cancer. Moreover, the data reveal that the presence of rs200996365, a SNP in high-linkage disequilibrium with rs8102137 residing in the center of a KLF5 motif, alters KLF5 binding to this genomic region. Through luciferase assays and CRISPR-Cas9 genome editing, a novel polymorphic intronic regulatory element controlling CCNE1 transcription is characterized. These studies uncover how a cancer-associated polymorphism mechanistically contributes to an increased predisposition for bladder cancer development. IMPLICATIONS: A polymorphic KLF5 binding site near the CCNE1 gene explains genetic risk identified through GWAS. *Mol Cancer Res*; 14(11); 1078-86. (c)2016 AACR.

Phelps, M. P., J. N. Bailey, et al. "CRISPR screen identifies the NCOR/HDAC3 complex as a major suppressor of differentiation in rhabdomyosarcoma." *Proc Natl Acad Sci U S A*. 2016 Dec 27;113(52):15090-15095. doi: [10.1073/pnas.1610270114](https://doi.org/10.1073/pnas.1610270114). Epub 2016 Dec 12.

Dysregulated gene expression resulting from abnormal epigenetic alterations including histone acetylation and deacetylation has been demonstrated to play an important role in driving tumor growth and progression. However, the mechanisms by which specific histone deacetylases (HDACs) regulate differentiation in solid tumors remains unclear. Using pediatric rhabdomyosarcoma (RMS) as a paradigm to elucidate the mechanism blocking differentiation in solid tumors, we identified HDAC3 as a major suppressor of myogenic differentiation from a high-efficiency Clustered regularly interspaced short palindromic repeats (CRISPR)-based phenotypic screen of class I and II HDAC genes. Detailed characterization of the HDAC3-knockout phenotype in vitro and in vivo using a tamoxifen-inducible CRISPR targeting strategy demonstrated that HDAC3 deacetylase activity and the formation of a functional complex with nuclear receptor corepressors (NCORs) were critical in restricting differentiation in RMS. The NCOR/HDAC3 complex specifically functions by blocking myoblast determination protein 1 (MYOD1)-mediated activation of myogenic differentiation. Interestingly, there was also a transient up-regulation of growth-promoting genes upon initial HDAC3 targeting, revealing a unique cancer-specific response to the forced transition from a neoplastic state to terminal differentiation. Our study applied modifications of CRISPR/CRISPR-associated endonuclease 9 (Cas9) technology to interrogate the function of essential cancer genes and pathways and has provided insights into cancer cell adaptation in response to altered differentiation status. Because current pan-HDAC inhibitors have shown disappointing results in clinical trials of solid tumors, therapeutic targets specific to HDAC3 function represent a promising option for differentiation therapy in malignant tumors with dysregulated HDAC3 activity.

Plouffe, S. W., Z. Meng, et al. "Characterization of Hippo Pathway Components by Gene Inactivation." *Mol Cell*. 2016 Dec 1;64(5):993-1008. doi: [10.1016/j.molcel.2016.10.034](https://doi.org/10.1016/j.molcel.2016.10.034).

The Hippo pathway is important for regulating tissue homeostasis, and its dysregulation has been implicated in human cancer. However, it is not well understood how the Hippo pathway becomes dysregulated because few mutations in core Hippo

pathway components have been identified. Therefore, much work in the Hippo field has focused on identifying upstream regulators, and a complex Hippo interactome has been identified. Nevertheless, it is not always clear which components are the most physiologically relevant in regulating YAP/TAZ. To provide an overview of important Hippo pathway components, we created knockout cell lines for many of these components and compared their relative contributions to YAP/TAZ regulation in response to a wide range of physiological signals. By this approach, we provide an overview of the functional importance of many Hippo pathway components and demonstrate NF2 and RHOA as important regulators of YAP/TAZ and TAOK1/3 as direct kinases for LATS1/2.

Polfus, L. M., R. K. Khajuria, et al. "Whole-Exome Sequencing Identifies Loci Associated with Blood Cell Traits and Reveals a Role for Alternative GFI1B Splice Variants in Human Hematopoiesis." *Am J Hum Genet*. 2016 Aug 4;99(2):481-8. doi: [10.1016/j.ajhg.2016.06.016](https://doi.org/10.1016/j.ajhg.2016.06.016).

Circulating blood cell counts and indices are important indicators of hematopoietic function and a number of clinical parameters, such as blood oxygen-carrying capacity, inflammation, and hemostasis. By performing whole-exome sequence association analyses of hematologic quantitative traits in 15,459 community-dwelling individuals, followed by in silico replication in up to 52,024 independent samples, we identified two previously undescribed coding variants associated with lower platelet count: a common missense variant in CPS1 (rs1047891, MAF = 0.33, discovery + replication $p = 6.38 \times 10^{-10}$) and a rare synonymous variant in GFI1B (rs150813342, MAF = 0.009, discovery + replication $p = 1.79 \times 10^{-27}$). By performing CRISPR/Cas9 genome editing in hematopoietic cell lines and follow-up targeted knockdown experiments in primary human hematopoietic stem and progenitor cells, we demonstrate an alternative splicing mechanism by which the GFI1B rs150813342 variant suppresses formation of a GFI1B isoform that preferentially promotes megakaryocyte differentiation and platelet production. These results demonstrate how unbiased studies of natural variation in blood cell traits can provide insight into the regulation of human hematopoiesis.

Pott, L. L., S. Hagemann, et al. "Eukaryotic elongation factor 2 is a prognostic marker and its kinase a potential therapeutic target in HCC." *Oncotarget*. 2017 Jan 2. doi: [10.18632/oncotarget.14447](https://doi.org/10.18632/oncotarget.14447).

Hepatocellular carcinoma is a cancer with increasing incidence and largely refractory to current anticancer drugs. Since Sorafenib, a multikinase

inhibitor has shown modest efficacy in advanced hepatocellular carcinoma additional treatments are highly needed. Protein phosphorylation via kinases is an important post-translational modification to regulate cell homeostasis including proliferation and apoptosis. Therefore kinases are valuable targets in cancer therapy. To this end we performed 2D differential gel electrophoresis and mass spectrometry analysis of phosphoprotein-enriched lysates of tumor and corresponding non-tumorous liver samples to detect differentially abundant phosphoproteins to screen for novel kinases as potential drug targets. We identified 34 differentially abundant proteins in phosphoprotein enriched lysates. Expression and distribution of the candidate protein eEF2 and its phosphorylated isoform was validated immunohistochemically on 78 hepatocellular carcinoma and non-tumorous tissue samples. Validation showed that total eEF2 and phosphorylated eEF2 at threonine 56 are prognostic markers for overall survival of HCC-patients. The activity of the regulating eEF2 kinase, compared between tumor and non-tumorous tissue lysates by in vitro kinase assays, is more than four times higher in tumor tissues. Functional analyzes regarding eEF2 kinase were performed in JHH5 cells with CRISPR/Cas9 mediated eEF2 kinase knock out. Proliferation and growth is decreased in eEF2 kinase knock out cells. CONCLUSION: eEF2 and phosphorylated eEF2 are prognostic markers for survival of hepatocellular carcinoma patients and the regulating eEF2 kinase is a potential drug target for tumor therapy.

Qaisar, N., S. Lin, et al. "A Critical Role for the Type I Interferon Receptor in Virus-Induced Autoimmune Diabetes in Rats." Diabetes. 2017 Jan;66(1):145-157. doi: 10.2337/db16-0462. Epub 2016 Oct 7.

The pathogenesis of human type 1 diabetes, characterized by immune-mediated damage of insulin-producing beta-cells of pancreatic islets, may involve viral infection. Essential components of the innate immune antiviral response, including type I interferon (IFN) and IFN receptor-mediated signaling pathways, are candidates for determining susceptibility to human type 1 diabetes. Numerous aspects of human type 1 diabetes pathogenesis are recapitulated in the LEW.1WR1 rat model. Diabetes can be induced in LEW.1WR1 weanling rats challenged with virus or with the viral mimetic polyinosinic:polycytidylic acid (poly I:C). We hypothesized that disrupting the cognate type I IFN receptor (type I IFN alpha/beta receptor [IFNAR]) to interrupt IFN signaling would prevent or delay the development of virus-induced diabetes. We generated IFNAR1 subunit-deficient LEW.1WR1 rats using CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-

associated protein 9) genome editing and confirmed functional disruption of the *Ifnar1* gene. IFNAR1 deficiency significantly delayed the onset and frequency of diabetes and greatly reduced the intensity of insulinitis after poly I:C treatment. The occurrence of Kilham rat virus-induced diabetes was also diminished in IFNAR1-deficient animals. These findings firmly establish that alterations in innate immunity influence the course of autoimmune diabetes and support the use of targeted strategies to limit or prevent the development of type 1 diabetes.

Quick, L., R. Young, et al. "Jak1-STAT3 Signals Are Essential Effectors of the USP6/TRE17 Oncogene in Tumorigenesis." Cancer Res. 2016 Sep 15;76(18):5337-47. doi: 10.1158/0008-5472.CAN-15-2391. Epub 2016 Jul 20.

Bone and soft tissue tumors (BSTT) are relatively poorly understood, hampering the development of effective therapies. Here we report a role for the ubiquitin-specific protease 6 (USP6)/TRE17 oncogene, which is overexpressed upon chromosome translocation in various human tumors, including aneurysmal bone cyst (ABC), and the related benign lesion nodular fasciitis. Ectopic expression of USP6 is known to drive formation of tumors, which recapitulate key features of ABC and nodular fasciitis; however, the identity of USP6's relevant substrates has been obscure. Here we report that the Jak1-STAT3 signaling pathway serves as an essential effector of USP6 in BSTT formation. We found that USP6 directly deubiquitinated Jak1, leading to its stabilization and activation of STAT3. The tumorigenic potential of USP6 was attenuated significantly by CRISPR-mediated deletion of Jak1 or STAT3, or by administration of a Jak family inhibitor. Analysis of primary clinical samples of nodular fasciitis confirmed the activation of a Jak1-STAT3 gene signature in vivo Together, our studies highlight Jak1 as the first identified substrate for USP6, and they offer a mechanistic rationale for the clinical investigation of Jak and STAT3 inhibitors as therapeutics for the treatment of bone and soft tissue tumors along with other neoplasms driven by USP6 overexpression. *Cancer Res*; 76(18); 5337-47. (c)2016 AACR.

Rauscher, B., F. Heigwer, et al. "GenomeCRISPR - a database for high-throughput CRISPR/Cas9 screens." Nucleic Acids Res. 2017 Jan 4;45(D1):D679-D686. doi: 10.1093/nar/gkw997. Epub 2016 Oct 26.

Over the past years, CRISPR/Cas9 mediated genome editing has developed into a powerful tool for modifying genomes in various organisms. In high-throughput screens, CRISPR/Cas9 mediated gene perturbations can be used for the systematic functional

analysis of whole genomes. Discoveries from such screens provide a wealth of knowledge about gene to phenotype relationships in various biological model systems. However, a database resource to query results efficiently has been lacking. To this end, we developed GenomeCRISPR (<http://genomecrispr.org>), a database for genome-scale CRISPR/Cas9 screens. Currently, GenomeCRISPR contains data on more than 550 000 single guide RNAs (sgRNA) derived from 84 different experiments performed in 48 different human cell lines, comprising all screens in human cells using CRISPR/Cas published to date. GenomeCRISPR provides data mining options and tools, such as gene or genomic region search. Phenotypic and genome track views allow users to investigate and compare the results of different screens, or the impact of different sgRNAs on the gene of interest. An Application Programming Interface (API) allows for automated data access and batch download. As more screening data will become available, we also aim at extending the database to include functional genomic data from other organisms and enable cross-species comparisons.

Ren, J., X. Liu, et al. "Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition." *Clin Cancer Res.* 2016 Nov 4. pii: [clincanres.1300.2016](https://doi.org/10.1158/1078-0432.CCR.1300.2016).

PURPOSE: Using gene-disrupted allogeneic T cells as universal effector cells provides an alternative to and potentially improves current chimeric antigen receptor (CAR) T cell therapy against cancers and infectious diseases. **EXPERIMENTAL DESIGN:** The CRISPR/Cas9 system has recently emerged as a simple and efficient way for multiplex genome engineering. By combining the lentiviral delivery of CAR and CRISPR RNA electroporation to co-introduce RNA encoding the Cas9 and gRNAs targeting endogenous TCR, beta-2 microglobulin (B2M) and PD1 simultaneously, to generate gene-disrupted allogeneic CAR T cells deficient of TCR, HLA class I molecule and PD1. **RESULTS:** The CRISPR gene-edited CAR T cells showed potent anti-tumor activities, both in vitro and in animal models and were as potent as non-gene edited CAR T cells. In addition the TCR and HLA class I double deficient T cells had reduced alloreactivity and did not cause graft-versus-host disease. Finally, simultaneous triple genome editing by adding the disruption of PD1 led to enhanced in vivo anti-tumor activity of the gene-disrupted CART cells. **CONCLUSIONS:** Gene-disrupted allogeneic CAR and TCR T cells could provide an alternative as a universal donor to autologous T cells, which carry difficulties and high production costs. Gene-disrupted CAR and TCR T cells with disabled checkpoint molecules may be potent effector cells against cancers and infectious

diseases.

Rivera-Torres, N., K. Banas, et al. "Insertional Mutagenesis by CRISPR/Cas9 Ribonucleoprotein Gene Editing in Cells Targeted for Point Mutation Repair Directed by Short Single-Stranded DNA Oligonucleotides." *PLoS One.* 2017 Jan 4;12(1):e0169350. doi: [10.1371/journal.pone.0169350](https://doi.org/10.1371/journal.pone.0169350). [eCollection 2017](https://doi.org/10.1371/journal.pone.0169350).

CRISPR/Cas9 and single-stranded DNA oligonucleotides (ssODNs) have been used to direct the repair of a single base mutation in human genes. Here, we examine a method designed to increase the precision of RNA guided genome editing in human cells by utilizing a CRISPR/Cas9 ribonucleoprotein (RNP) complex to initiate DNA cleavage. The RNP is assembled in vitro and induces a double stranded break at a specific site surrounding the mutant base designated for correction by the ssODN. We use an integrated mutant eGFP gene, bearing a single base change rendering the expressed protein nonfunctional, as a single copy target in HCT 116 cells. We observe significant gene correction activity of the mutant base, promoted by the RNP and single-stranded DNA oligonucleotide with validation through genotypic and phenotypic readout. We demonstrate that all individual components must be present to obtain successful gene editing. Importantly, we examine the genotype of individually sorted corrected and uncorrected clonally expanded cell populations for the mutagenic footprint left by the action of these gene editing tools. While the DNA sequence of the corrected population is exact with no adjacent sequence modification, the uncorrected population exhibits heterogeneous mutagenicity with a wide variety of deletions and insertions surrounding the target site. We designate this type of DNA aberration as on-site mutagenicity. Analyses of two clonal populations bearing specific DNA insertions surrounding the target site, indicate that point mutation repair has occurred at the level of the gene. The phenotype, however, is not rescued because a section of the single-stranded oligonucleotide has been inserted altering the reading frame and generating truncated proteins. These data illustrate the importance of analysing mutagenicity in uncorrected cells. Our results also form the basis of a simple model for point mutation repair directed by a short single-stranded DNA oligonucleotides and CRISPR/Cas9 ribonucleoprotein complex.

Rosenbluh, J., J. Mercer, et al. "Genetic and Proteomic Interrogation of Lower Confidence Candidate Genes Reveals Signaling Networks in beta-Catenin-Active Cancers." *Cell Syst.* 2016 Sep 28;3(3):302-316.e4. doi: [10.1016/j.cels.2016.09.001](https://doi.org/10.1016/j.cels.2016.09.001).

Genome-scale expression studies and

comprehensive loss-of-function genetic screens have focused almost exclusively on the highest confidence candidate genes. Here, we describe a strategy for characterizing the lower confidence candidates identified by such approaches. We interrogated 177 genes that we classified as essential for the proliferation of cancer cells exhibiting constitutive beta-catenin activity and integrated data for each of the candidates, derived from orthogonal short hairpin RNA (shRNA) knockdown and clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9-mediated gene editing knockout screens, to yield 69 validated genes. We then characterized the relationships between sets of these genes using complementary assays: medium-throughput stable isotope labeling by amino acids in cell culture (SILAC)-based mass spectrometry, yielding 3,639 protein-protein interactions, and a CRISPR-mediated pairwise double knockout screen, yielding 375 combinations exhibiting greater- or lesser-than-additive phenotypic effects indicating genetic interactions. These studies identify previously unreported regulators of beta-catenin, define functional networks required for the survival of beta-catenin-active cancers, and provide an experimental strategy that may be applied to define other signaling networks.

Sanjana, N. E., J. Wright, et al. "High-resolution interrogation of functional elements in the noncoding genome." *Science*. 2016 Sep 30;353(6307):1545-1549.

The noncoding genome affects gene regulation and disease, yet we lack tools for rapid identification and manipulation of noncoding elements. We developed a CRISPR screen using ~18,000 single guide RNAs targeting >700 kilobases surrounding the genes NF1, NF2, and CUL3, which are involved in BRAF inhibitor resistance in melanoma. We find that noncoding locations that modulate drug resistance also harbor predictive hallmarks of noncoding function. With a subset of regions at the CUL3 locus, we demonstrate that engineered mutations alter transcription factor occupancy and long-range and local epigenetic environments, implicating these sites in gene regulation and chemotherapeutic resistance. Through our expansion of the potential of pooled CRISPR screens, we provide tools for genomic discovery and for elucidating biologically relevant mechanisms of gene regulation.

Sano, R., T. Nakajima, et al. "Epithelial Expression of Human ABO Blood Group Genes Is Dependent upon a Downstream Regulatory Element Functioning through an Epithelial Cell-specific Transcription Factor, Elf5." *J Biol Chem*. 2016 Oct 21;291(43):22594-22606. [Epub 2016 Sep 1.](#)

The human ABO blood group system is of great importance in blood transfusion and organ transplantation. The ABO system is composed of complex carbohydrate structures that are biosynthesized by A- and B-transferases encoded by the ABO gene. However, the mechanisms regulating ABO gene expression in epithelial cells remain obscure. On the basis of DNase I-hypersensitive sites in and around ABO in epithelial cells, we prepared reporter plasmid constructs including these sites. Subsequent luciferase assays and histone modifications indicated a novel positive regulatory element, designated the +22.6-kb site, downstream from ABO, and this was shown to enhance ABO promoter activity in an epithelial cell-specific manner. Expression of ABO and B-antigen was reduced in gastric cancer KATOIII cells by biallelic deletion of the +22.6-kb site using the CRISPR/Cas9 system. Electrophoretic mobility shift assay and chromatin immunoprecipitation assay demonstrated that the site bound to an epithelial cell-specific transcription factor, Elf5. Mutation of the Ets binding motifs to abrogate binding of this factor reduced the regulatory activity of the +22.6-kb site. Furthermore, ELF5 knockdown with shRNA reduced both endogenous transcription from ABO and B-antigen expression in KATOIII cells. Thus, Elf5 appeared to be involved in the enhancer potential of the +22.6-kb site. These results support the contention that ABO expression is dependent upon a downstream positive regulatory element functioning through a tissue-restricted transcription factor, Elf5, in epithelial cells.

Sansregret, L., J. O. Patterson, et al. "APC/C Dysfunction Limits Excessive Cancer Chromosomal Instability." *Cancer Discov*. 2017 Jan 9. doi: [10.1158/2159-8290.CD-16-0645](https://doi.org/10.1158/2159-8290.CD-16-0645).

Intercellular heterogeneity, exacerbated by chromosomal instability (CIN), fosters tumor heterogeneity and drug resistance. However, extreme CIN correlates with improved cancer outcome, suggesting that karyotypic diversity required to adapt to selection pressures might be balanced in tumors against the risk of excessive instability. Here, we used a functional genomics screen, genome editing, and pharmacologic approaches to identify CIN-survival factors in diploid cells. We find partial anaphase-promoting complex/cyclosome (APC/C) dysfunction lengthens mitosis, suppresses pharmacologically induced chromosome segregation errors, and reduces naturally occurring lagging chromosomes in cancer cell lines or following tetraploidization. APC/C impairment caused adaptation to MPS1 inhibitors, revealing a likely resistance mechanism to therapies targeting the spindle assembly checkpoint. Finally, CRISPR-mediated introduction of cancer somatic

mutations in the APC/C subunit cancer driver gene CDC27 reduces chromosome segregation errors, whereas reversal of an APC/C subunit nonsense mutation increases CIN. Subtle variations in mitotic duration, determined by APC/C activity, influence the extent of CIN, allowing cancer cells to dynamically optimize fitness during tumor evolution. SIGNIFICANCE: We report a mechanism whereby cancers balance the evolutionary advantages associated with CIN against the fitness costs caused by excessive genome instability, providing insight into the consequence of CDC27 APC/C subunit driver mutations in cancer. Lengthening of mitosis through APC/C modulation may be a common mechanism of resistance to cancer therapeutics that increase chromosome segregation errors. *Cancer Discov*; 7(2); 1-16. (c)2017 AACR.

Sayin, V. I. and T. Papagiannakopoulos "Application of CRISPR-mediated genome engineering in cancer research." *Cancer Lett.* 2017 Feb 28;387:10-17. doi: [10.1016/j.canlet.2016.03.029](https://doi.org/10.1016/j.canlet.2016.03.029). Epub 2016 Mar 18.

Cancer is a multistep process that arises from a series of genetic and epigenetic events. With recent technological advances there has been a burst in genome sequencing and epigenetic studies revealing a plethora of alterations that may contribute to cancer. However, the great challenge for the cancer research community is the systematic functional characterization of these genetic and epigenetic events to assess their role in cancer initiation and progression. Recent advances in genome engineering using CRISPR/Cas9, an ancient bacterial immune-like system, have revolutionized cancer genetics. Here we highlight the breakthroughs in the effective use of these novel genome-editing techniques, and we discuss the challenges and potential applications of these tools for cancer biology.

Schmidt, J. C., A. J. Zaugg, et al. "Live Cell Imaging Reveals the Dynamics of Telomerase Recruitment to Telomeres." *Cell.* 2016 Aug 25;166(5):1188-1197.e9. doi: [10.1016/j.cell.2016.07.033](https://doi.org/10.1016/j.cell.2016.07.033). Epub 2016 Aug 11.

Telomerase maintains genome integrity by adding repetitive DNA sequences to the chromosome ends in actively dividing cells, including 90% of all cancer cells. Recruitment of human telomerase to telomeres occurs during S-phase of the cell cycle, but the molecular mechanism of the process is only partially understood. Here, we use CRISPR genome editing and single-molecule imaging to track telomerase trafficking in nuclei of living human cells. We demonstrate that telomerase uses three-dimensional diffusion to search for telomeres, probing each telomere thousands of times each S-phase but only rarely forming a stable association. Both the

transient and stable association events depend on the direct interaction of the telomerase protein TERT with the telomeric protein TPP1. Our results reveal that telomerase recruitment to telomeres is driven by dynamic interactions between the rapidly diffusing telomerase and the chromosome end.

Senis, E., S. Mockenhaupt, et al. "TALEN/CRISPR-mediated engineering of a promoterless anti-viral RNAi hairpin into an endogenous miRNA locus." *Nucleic Acids Res.* 2017 Jan 9;45(1):e3. doi: [10.1093/nar/gkw805](https://doi.org/10.1093/nar/gkw805). Epub 2016 Sep 9.

Successful RNAi applications depend on strategies allowing robust and persistent expression of minimal gene silencing triggers without perturbing endogenous gene expression. Here, we propose a novel avenue which is integration of a promoterless shmiRNA, i.e. a shRNA embedded in a micro-RNA (miRNA) scaffold, into an engineered genomic miRNA locus. For proof-of-concept, we used TALE or CRISPR/Cas9 nucleases to site-specifically integrate an anti-hepatitis C virus (HCV) shmiRNA into the liver-specific miR-122/hcr locus in hepatoma cells, with the aim to obtain cellular clones that are genetically protected against HCV infection. Using reporter assays, Northern blotting and qRT-PCR, we confirmed anti-HCV shmiRNA expression as well as miR-122 integrity and functionality in selected cellular progeny. Moreover, we employed a comprehensive battery of PCR, cDNA/miRNA profiling and whole genome sequencing analyses to validate targeted integration of a single shmiRNA molecule at the expected position, and to rule out deleterious effects on the genomes or transcriptomes of the engineered cells. Importantly, a subgenomic HCV replicon and a full-length reporter virus, but not a Dengue virus control, were significantly impaired in the modified cells. Our original combination of DNA engineering and RNAi expression technologies benefits numerous applications, from miRNA, genome and transgenesis research, to human gene therapy.

Simonik, E. A., Y. Cai, et al. "LIM-Only Protein 4 (LMO4) and LIM Domain Binding Protein 1 (LDB1) Promote Growth and Metastasis of Human Head and Neck Cancer (LMO4 and LDB1 in Head and Neck Cancer)." *PLoS One.* 2016 Oct 25;11(10):e0164804. doi: [10.1371/journal.pone.0164804](https://doi.org/10.1371/journal.pone.0164804). eCollection 2016.

Squamous cell carcinoma of the head and neck (HNSCC) accounts for more than 300,000 deaths worldwide per year as a consequence of tumor cell invasion of adjacent structures or metastasis. LIM-only protein 4 (LMO4) and LIM-domain binding protein 1 (LDB1), two directly interacting transcriptional adaptors that have important roles in normal epithelial cell differentiation, have been associated with

increased metastasis, decreased differentiation, and shortened survival in carcinoma of the breast. Here, we implicate two LDB1-binding proteins, single-stranded binding protein 2 (SSBP2) and 3 (SSBP3), in controlling LMO4 and LDB1 protein abundance in HNSCC and in regulating specific tumor cell functions in this disease. First, we found that the relative abundance of LMO4, LDB1, and the two SSBPs correlated very significantly in a panel of human HNSCC cell lines. Second, expression of these proteins in tumor primaries and lymph nodes involved by metastasis were concordant in 3 of 3 sets of tissue. Third, using a Matrigel invasion and organotypic reconstruct assay, CRISPR/Cas9-mediated deletion of LDB1 in the VU-SCC-1729 cell line, which is highly invasive of basement membrane and cellular monolayers, reduced tumor cell invasiveness and migration, as well as proliferation on tissue culture plastic. Finally, inactivation of the LDB1 gene in these cells decreased growth and vascularization of xenografted human tumor cells in vivo. These data show that LMO4, LDB1, and SSBP2 and/or SSBP3 regulate metastasis, proliferation, and angiogenesis in HNSCC and provide the first evidence that SSBPs control LMO4 and LDB1 protein abundance in a cancer context.

Singer, M., C. Wang, et al. "A Distinct Gene Module for Dysfunction Uncoupled from Activation in Tumor-Infiltrating T Cells." *Cell*. 2016 Sep 8;166(6):1500-1511.e9. doi: 10.1016/j.cell.2016.08.052.

Reversing the dysfunctional T cell state that arises in cancer and chronic viral infections is the focus of therapeutic interventions; however, current therapies are effective in only some patients and some tumor types. To gain a deeper molecular understanding of the dysfunctional T cell state, we analyzed population and single-cell RNA profiles of CD8(+) tumor-infiltrating lymphocytes (TILs) and used genetic perturbations to identify a distinct gene module for T cell dysfunction that can be uncoupled from T cell activation. This distinct dysfunction module is downstream of intracellular metallothioneins that regulate zinc metabolism and can be identified at single-cell resolution. We further identify Gata-3, a zinc-finger transcription factor in the dysfunctional module, as a regulator of dysfunction, and we use CRISPR-Cas9 genome editing to show that it drives a dysfunctional phenotype in CD8(+) TILs. Our results open novel avenues for targeting dysfunctional T cell states while leaving activation programs intact.

Soh, C. L. and D. Huangfu "CRISPR/Cas9-Mediated Mutagenesis of Human Pluripotent Stem Cells in Defined Xeno-Free E8 Medium." *Methods Mol Biol*. 2017;1498:57-78.

The recent advent of engineered nucleases including the CRISPR/Cas9 system has greatly facilitated genome manipulation in human pluripotent stem cells (hPSCs). In addition to facilitating hPSC-based disease studies, the application of genome engineering in hPSCs has also opened up new avenues for cell replacement therapy. To improve consistency and reproducibility of hPSC-based studies, and to meet the safety and regulatory requirements for clinical translation, it is necessary to use a defined, xeno-free cell culture system. This chapter describes protocols for CRISPR/Cas9 genome editing in an inducible Cas9 hPSC-based system, using cells cultured in chemically defined, xeno-free E8 Medium on a recombinant human vitronectin substrate. We detail procedures for the design and transfection of CRISPR guide RNAs, colony selection, and the expansion and validation of clonal mutant lines, all within this fully defined culture condition. These methods may be applied to a wide range of genome-engineering applications in hPSCs, including those that utilize different types of site-specific nucleases such as zinc finger nucleases (ZFNs) and TALENs, and form a closer step towards clinical utility of these cells.

Song, C. Q., Y. Li, et al. "Genome-wide CRISPR Screen Identifies Regulators of MAPK as Suppressors of Liver Tumors in Mice." *Gastroenterology*. 2016 Dec 9. pii: S0016-5085(16)35462-2. doi: 10.1053/j.gastro.2016.12.002.

BACKGROUND & AIMS: It has been a challenge to identify liver tumor suppressors or oncogenes due to the genetic heterogeneity of these tumors. We performed a genome-wide screen to identify suppressors of liver tumor formation in mice, using CRISPR-mediated genome editing. **METHODS:** We performed a genome-wide CRISPR/Cas9-based knockout screen of P53-null mouse embryonic liver progenitor cells that overexpressed MYC. We infected p53-/-;Myc;Cas9 hepatocytes with the mGeCKOa lentiviral library of 67,000 single-guide RNAs (sgRNAs), targeting 20,611 mouse genes, and transplanted the transduced cells subcutaneously into nude mice. Within 1 month, all the mice that received the sgRNA library developed subcutaneous tumors. We performed high-throughput sequencing of tumor DNA and identified sgRNAs increased at least 8-fold compared to the initial cell pool. To validate the top 10 candidate tumor suppressors from this screen, we collected data from patients with hepatocellular carcinoma (HCC) using the Cancer Genome Atlas and COSMIC databases. We used CRISPR to inactivate candidate tumor suppressor genes in p53-/-;Myc;Cas9 cells and transplanted them subcutaneously into nude mice; tumor formation was monitored and tumors were analyzed by histology and immunohistochemistry.

Mice with liver-specific disruption of p53 were given hydrodynamic tail-vein injections of plasmids encoding Myc and sgRNA/Cas9 designed to disrupt candidate tumor suppressors; growth of tumors and metastases was monitored. We compared gene expression profiles of liver cells with vs without tumor suppressor gene disrupted by sgRNA/Cas9. Genes found to be upregulated following tumor suppressor loss were examined in liver cancer cell lines; their expression was knocked down using small hairpin RNAs, and tumor growth was examined in nude mice. Effects of the MEK inhibitors AZD6244, U0126, and trametinib, or the multi-kinase inhibitor sorafenib, were examined in human and mouse HCC cell lines. RESULTS: We identified 4 candidate liver tumor suppressor genes not previously associated with liver cancer (Nf1, Plxnb1, Flrt2, and B9d1). CRISPR-mediated knockout of Nf1, a negative regulator of RAS, accelerated liver tumor formation in mice. Loss of Nf1 or activation of RAS upregulated the liver progenitor cell markers HMGA2 and SOX9. RAS pathway inhibitors suppressed the activation of the Hmga2 and Sox9 genes that resulted from loss of Nf1 or oncogenic activation of RAS. Knockdown of HMGA2 delayed formation of xenograft tumors from cells that expressed oncogenic RAS. In human HCCs, low levels of NF1 mRNA or high levels of HMGA2 mRNA were associated with shorter patient survival time. Liver cancer cells with inactivation of Plxnb1, Flrt2, and B9d1 formed more tumors in mice and had increased levels of MAPK phosphorylation. CONCLUSIONS: Using a CRISPR-based strategy, we identified Nf1, Plxnb1, Flrt2, and B9d1 as suppressors of liver tumor formation. We validated the observation that RAS signaling, via MAPK, contributes to formation of liver tumors in mice. We associated decreased levels of NF1 and increased levels of its downstream protein HMGA2 with survival times of patients with HCC. Strategies to inhibit or reduce HMGA2 might be developed to treat patients with liver cancer.

Song, Y., A. Ray, et al. "Targeting proteasome ubiquitin receptor Rpn13 in multiple myeloma." *Leukemia*. 2016 Sep;30(9):1877-86. doi: [10.1038/leu.2016.97](https://doi.org/10.1038/leu.2016.97). Epub 2016 Apr 27.

Proteasome inhibitor bortezomib is an effective therapy for relapsed and newly diagnosed multiple myeloma (MM); however, dose-limiting toxicities and the development of resistance can limit its long-term utility. Recent research has focused on targeting ubiquitin receptors upstream of 20S proteasome, with the aim of generating less toxic therapies. Here we show that 19S proteasome-associated ubiquitin receptor Rpn13 is more highly expressed in MM cells than in normal plasma cells.

Rpn13-siRNA (small interfering RNA) decreases MM cell viability. A novel agent RA190 targets Rpn13 and inhibits proteasome function, without blocking the proteasome activity or the 19S deubiquitylating activity. CRISPR/Cas9 Rpn13-knockout demonstrates that RA190-induced activity is dependent on Rpn13. RA190 decreases viability in MM cell lines and patient MM cells, inhibits proliferation of MM cells even in the presence of bone marrow stroma and overcomes bortezomib resistance. Anti-MM activity of RA190 is associated with induction of caspase-dependent apoptosis and unfolded protein response-related apoptosis. MM xenograft model studies show that RA190 is well tolerated, inhibits tumor growth and prolongs survival. Combining RA190 with bortezomib, lenalidomide or pomalidomide induces synergistic anti-MM activity. Our preclinical data validates targeting Rpn13 to overcome bortezomib resistance, and provides the framework for clinical evaluation of Rpn13 inhibitors, alone or in combination, to improve patient outcome in MM.

Sosale, N. G., Ivanovska, II, et al. "'Marker of Self' CD47 on lentiviral vectors decreases macrophage-mediated clearance and increases delivery to SIRPA-expressing lung carcinoma tumors." *Mol Ther Methods Clin Dev*. 2016 Dec 7;3:16080. doi: [10.1038/mtm.2016.80](https://doi.org/10.1038/mtm.2016.80). eCollection 2016.

Lentiviruses infect many cell types and are now widely used for gene delivery in vitro, but in vivo uptake of these foreign vectors by macrophages is a limitation. Lentivectors are produced here from packaging cells that overexpress "Marker of Self" CD47, which inhibits macrophage uptake of cells when phagocytic factors are also displayed. Single particle analyses show "hCD47-Lenti" display properly oriented human-CD47 for interactions with the macrophage's inhibitory receptor SIRPA. Macrophages derived from human and NOD/SCID/Il2rg^{-/-} (NSG) mice show a SIRPA-dependent decrease in transduction, i.e., transgene expression, by hCD47-Lenti compared to control Lenti. Consistent with known "Self" signaling pathways, macrophage transduction by control Lenti is decreased by drug inhibition of Myosin-II to the same levels as hCD47-Lenti. In contrast, human lung carcinoma cells express SIRPA and use it to enhance transduction by hCD47-Lenti- as illustrated by more efficient gene deletion using CRISPR/Cas9. Intravenous injection of hCD47-Lenti into NSG mice shows hCD47 prolongs circulation, unless a blocking anti-SIRPA is preinjected. In vivo transduction of spleen and liver macrophages also decreases for hCD47-Lenti while transduction of lung carcinoma xenografts increases. hCD47 could be useful when macrophage uptake is limiting on other viral vectors that are emerging in

cancer treatments (e.g., Measles glycoprotein-pseudotyped lentivectors) and also in targeting various SIRPA-expressing tumors such as glioblastomas.

Sotiriou, S. K., I. Kamileri, et al. "Mammalian RAD52 Functions in Break-Induced Replication Repair of Collapsed DNA Replication Forks." *Mol Cell*. 2016 Dec 15;64(6):1127-1134. doi: [10.1016/j.molcel.2016.10.038](https://doi.org/10.1016/j.molcel.2016.10.038).

Human cancers are characterized by the presence of oncogene-induced DNA replication stress (DRS), making them dependent on repair pathways such as break-induced replication (BIR) for damaged DNA replication forks. To better understand BIR, we performed a targeted siRNA screen for genes whose depletion inhibited G1 to S phase progression when oncogenic cyclin E was overexpressed. RAD52, a gene dispensable for normal development in mice, was among the top hits. In cells in which fork collapse was induced by oncogenes or chemicals, the Rad52 protein localized to DRS foci. Depletion of Rad52 by siRNA or knockout of the gene by CRISPR/Cas9 compromised restart of collapsed forks and led to DNA damage in cells experiencing DRS. Furthermore, in cancer-prone, heterozygous APC mutant mice, homozygous deletion of the Rad52 gene suppressed tumor growth and prolonged lifespan. We therefore propose that mammalian RAD52 facilitates repair of collapsed DNA replication forks in cancer cells.

Stanley, S. E., D. L. Gable, et al. "Loss-of-function mutations in the RNA biogenesis factor NAF1 predispose to pulmonary fibrosis-emphysema." *Sci Transl Med*. 2016 Aug 10;8(351):351ra107. doi: [10.1126/scitranslmed.aaf7837](https://doi.org/10.1126/scitranslmed.aaf7837).

Chronic obstructive pulmonary disease and pulmonary fibrosis have been hypothesized to represent premature aging phenotypes. At times, they cluster in families, but the genetic basis is not understood. We identified rare, frameshift mutations in the gene for nuclear assembly factor 1, NAF1, a box H/ACA RNA biogenesis factor, in pulmonary fibrosis-emphysema patients. The mutations segregated with short telomere length, low telomerase RNA levels, and extrapulmonary manifestations including myelodysplastic syndrome and liver disease. A truncated NAF1 was detected in cells derived from patients, and, in cells in which the frameshift mutation was introduced by genome editing, telomerase RNA levels were reduced. The mutant NAF1 lacked a conserved carboxyl-terminal motif, which we show is required for nuclear localization. To understand the disease mechanism, we used CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein-9 nuclease) to generate Naf1(+/-) mice and found that they had half the levels

of telomerase RNA. Other box H/ACA RNA levels were also decreased, but rRNA pseudouridylation, which is guided by snoRNAs, was intact. Moreover, first-generation Naf1(+/-) mice showed no evidence of ribosomal pathology. Our data indicate that disease in NAF1 mutation carriers is telomere-mediated; they show that NAF1 haploinsufficiency selectively disturbs telomere length homeostasis by decreasing the levels of telomerase RNA while sparing rRNA pseudouridylation.

Steinhart, Z., Z. Pavlovic, et al. "Genome-wide CRISPR screens reveal a Wnt-FZD5 signaling circuit as a druggable vulnerability of RNF43-mutant pancreatic tumors." *Nat Med*. 2017 Jan;23(1):60-68. doi: [10.1038/nm.4219](https://doi.org/10.1038/nm.4219). Epub 2016 Nov 21.

Forward genetic screens with CRISPR-Cas9 genome editing enable high-resolution detection of genetic vulnerabilities in cancer cells. We conducted genome-wide CRISPR-Cas9 screens in RNF43-mutant pancreatic ductal adenocarcinoma (PDAC) cells, which rely on Wnt signaling for proliferation. Through these screens, we discovered a unique requirement for a Wnt signaling circuit: engaging FZD5, one of the ten Frizzled receptors encoded in the human genome. Our results uncover an underappreciated level of context-dependent specificity at the Wnt receptor level. We further derived a panel of recombinant antibodies that reports the expression of nine FZD proteins and confirms that FZD5 functional specificity cannot be explained by protein expression patterns. Additionally, antibodies that specifically bind FZD5 and FZD8 robustly inhibited the growth of RNF43-mutant PDAC cells grown in vitro and as xenografts in vivo, providing orthogonal support for the functional specificity observed genetically. Proliferation of a patient-derived PDAC cell line harboring an RNF43 variant was also selectively inhibited by the FZD5 antibodies, further demonstrating their use as a potential targeted therapy. Tumor organoid cultures from colorectal carcinoma patients that carried RNF43 mutations were also sensitive to the FZD5 antibodies, highlighting the potential generalizability of these findings beyond PDAC. Our results show that CRISPR-based genetic screens can be leveraged to identify and validate cell surface targets for antibody development and therapy.

Tahara, S., S. Nojima, et al. "S100A4 accelerates the proliferation and invasion of endometrioid carcinoma and is associated with the "MELF" pattern." *Cancer Sci*. 2016 Sep;107(9):1345-52. doi: [10.1111/cas.12999](https://doi.org/10.1111/cas.12999). Epub 2016 Aug 12.

Endometrioid carcinoma (EC) is one of the most common malignancies of the female genital system. Although the behavior of EC ranges from an

excellent prognosis to aggressive disease with a poor outcome, the factors that determine its diversity have not been determined. Here, we show that S100A4, a calcium-binding protein of the EF-hand type, is correlated with the proliferation and invasion ability of EC. We demonstrated previously that EC cells with high aldehyde dehydrogenase (ALDH) activity were more tumorigenic than ALDH-lo cells. Screening by shotgun proteomics demonstrated that the expression level of S100A4 in ALDH-hi EC cells was significantly higher than that in ALDH-lo cells. S100A4-knockout cells generated by the CRISPR/Cas9 system showed reduced proliferation and invasion. These cells showed impaired AKT phosphorylation and matrix metalloproteinase-2 activation, accounting for their impaired proliferation and invasion, respectively. Furthermore, in clinical EC samples, elevated expression of S100A4 was highly related to myometrial and lymphatic invasion in well to moderately differentiated EC. Notably, strong and diffuse expression of S100A4 was observed in tumor tissues with a microcystic, elongated and fragmented ("MELF") pattern, which is associated with a highly invasive EC phenotype. Collectively, our results demonstrate not only that high expression of S100A4 contributes to an aggressive phenotype of EC, but also that its elevated expression is closely related to the MELF histopathological pattern.

Tang, K. J., J. D. Conzanzo, et al. "Focal Adhesion Kinase Regulates the DNA Damage Response and Its Inhibition Radiosensitizes Mutant KRAS Lung Cancer." *Clin Cancer Res.* 2016 Dec 1;22(23):5851-5863. Epub 2016 May 24.

PURPOSE: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide due to the limited availability of effective therapeutic options. For instance, there are no effective strategies for NSCLCs that harbor mutant KRAS, the most commonly mutated oncogene in NSCLC. Thus, our purpose was to make progress toward the generation of a novel therapeutic strategy for NSCLC. **EXPERIMENTAL DESIGN:** We characterized the effects of suppressing focal adhesion kinase (FAK) by RNA interference (RNAi), CRISPR/CAS9 gene editing or pharmacologic approaches in NSCLC cells and in tumor xenografts. In addition, we tested the effects of suppressing FAK in association with ionizing radiation (IR), a standard-of-care treatment modality. **RESULTS:** FAK is a critical requirement of mutant KRAS NSCLC cells. With functional experiments, we also found that, in mutant KRAS NSCLC cells, FAK inhibition resulted in persistent DNA damage and susceptibility to exposure to IR. Accordingly, administration of IR to FAK-null tumor xenografts causes a profound antitumor effect in vivo

CONCLUSIONS: FAK is a novel regulator of DNA damage repair in mutant KRAS NSCLC and its pharmacologic inhibition leads to radiosensitizing effects that could be beneficial in cancer therapy. Our results provide a framework for the rationale clinical testing of FAK inhibitors in NSCLC patients. *Clin Cancer Res.* 22(23); 5851-63. (c)2016 AACR.

Tao, L., J. Zhang, et al. "Frizzled proteins are colonic epithelial receptors for *C. difficile* toxin B." *Nature.* 2016 Oct 20;538(7625):350-355. doi: 10.1038/nature19799. Epub 2016 Sep 28.

Clostridium difficile toxin B (TcdB) is a critical virulence factor that causes diseases associated with *C. difficile* infection. Here we carried out CRISPR-Cas9-mediated genome-wide screens and identified the members of the Wnt receptor frizzled family (FZDs) as TcdB receptors. TcdB binds to the conserved Wnt-binding site known as the cysteine-rich domain (CRD), with the highest affinity towards FZD1, 2 and 7. TcdB competes with Wnt for binding to FZDs, and its binding blocks Wnt signalling. FZD1/2/7 triple-knockout cells are highly resistant to TcdB, and recombinant FZD2-CRD prevented TcdB binding to the colonic epithelium. Colonic organoids cultured from FZD7-knockout mice, combined with knockdown of FZD1 and 2, showed increased resistance to TcdB. The colonic epithelium in FZD7-knockout mice was less susceptible to TcdB-induced tissue damage in vivo. These findings establish FZDs as physiologically relevant receptors for TcdB in the colonic epithelium.

Tschaharganeh, D. F., S. W. Lowe, et al. "Using CRISPR/Cas to study gene function and model disease in vivo." *FEBS J.* 2016 Sep;283(17):3194-203. doi: 10.1111/febs.13750. Epub 2016 Jun 16.

The recent discovery of the CRISPR/Cas system and repurposing of this technology to edit a variety of different genomes have revolutionized an array of scientific fields, from genetics and translational research, to agriculture and bioproduction. In particular, the prospect of rapid and precise genome editing in laboratory animals by CRISPR/Cas has generated an immense interest in the scientific community. Here we review current in vivo applications of CRISPR/Cas and how this technology can improve our knowledge of gene function and our understanding of biological processes in animal models.

Tzelepis, K., H. Koike-Yusa, et al. "A CRISPR Dropout Screen Identifies Genetic Vulnerabilities and Therapeutic Targets in Acute Myeloid Leukemia." *Cell Rep.* 2016 Oct 18;17(4):1193-1205. doi: 10.1016/j.celrep.2016.09.079.

Acute myeloid leukemia (AML) is an aggressive cancer with a poor prognosis, for which mainstream treatments have not changed for decades. To identify additional therapeutic targets in AML, we optimize a genome-wide clustered regularly interspaced short palindromic repeats (CRISPR) screening platform and use it to identify genetic vulnerabilities in AML cells. We identify 492 AML-specific cell-essential genes, including several established therapeutic targets such as DOT1L, BCL2, and MEN1, and many other genes including clinically actionable candidates. We validate selected genes using genetic and pharmacological inhibition, and chose KAT2A as a candidate for downstream study. KAT2A inhibition demonstrated anti-AML activity by inducing myeloid differentiation and apoptosis, and suppressed the growth of primary human AMLs of diverse genotypes while sparing normal hemopoietic stem-progenitor cells. Our results propose that KAT2A inhibition should be investigated as a therapeutic strategy in AML and provide a large number of genetic vulnerabilities of this leukemia that can be pursued in downstream studies.

Vafai, S. B., E. Mevers, et al. "Natural Product Screening Reveals Naphthoquinone Complex I Bypass Factors." *PLoS One*. 2016 Sep 13;11(9):e0162686. doi: [10.1371/journal.pone.0162686](https://doi.org/10.1371/journal.pone.0162686). eCollection 2016.

Deficiency of mitochondrial complex I is encountered in both rare and common diseases, but we have limited therapeutic options to treat this lesion to the oxidative phosphorylation system (OXPHOS). Idebenone and menadione are redox-active molecules capable of rescuing OXPHOS activity by engaging complex I-independent pathways of entry, often referred to as "complex I bypass." In the present study, we created a cellular model of complex I deficiency by using CRISPR genome editing to knock out *Ndufa9* in mouse myoblasts, and utilized this cell line to develop a high-throughput screening platform for novel complex I bypass factors. We screened a library of ~40,000 natural product extracts and performed bioassay-guided fractionation on a subset of the top scoring hits. We isolated four plant-derived 1,4-naphthoquinone complex I bypass factors with structural similarity to menadione: chimaphilin and 3-chloro-chimaphilin from *Chimaphila umbellata* and dehydro-alpha-lapachone and dehydroiso-alpha-lapachone from *Stereospermum euphoroides*. We also tested a small number of structurally related naphthoquinones from commercial sources and identified two additional compounds with complex I bypass activity: 2-methoxy-1,4-naphthoquinone and 2-methoxy-3-methyl-1,4-naphthoquinone. The six novel complex I bypass factors reported here expand this class of molecules and will be useful as tool

compounds for investigating complex I disease biology.

Valdiani, A., D. Talei, et al. "Genoproteomics-assisted improvement of *Andrographis paniculata*: toward a promising molecular and conventional breeding platform for autogamous plants affecting the pharmaceutical industry." *Crit Rev Biotechnol*. 2017 Jan 3:1-14. doi: [10.1080/07388551.2016.1260525](https://doi.org/10.1080/07388551.2016.1260525).

Andrographis paniculata (Burm. f.) Wall. ex Nees. (AP) is a hermaphroditic, self-compatible, and habitual inbreeding plant. Its main bioactive component is andrographolide, which is capable of inducing autophagic cell death in some human cancer cells and helps fight HIV/AIDS. Increasing the andrographolide content by investigating the genetic mechanisms controlling its biosynthesis in order to improve and develop high-yielding cultivars are the main breeding targets for AP. However, there might exist some limitations or barriers for crossability within AP accessions. Recently, this problem was addressed in AP by using a combination of crossbreeding and biotechnology-aided genetic methods. This review emphasizes that development of a breeding platform in a hard-to-breed plant, such as AP, requires the involvement of a broad range of methods from classical genetics to molecular breeding. To this end, a phenological stage (for example, flowering and stigma development) can be simplified to a quantitative morphological trait (for example, bud or stigma length) to be used as an index to express the highest level of receptivity in order to manage outcrossing. The outcomes of the basic crossability research can be then employed in diallel mating and crossbreeding. This review explains how genomic data could produce useful information regarding genetic distance and its influence on the crossability of AP accessions. Our review indicates that co-dominant DNA markers, such as microsatellites, are also capable of resolving the evolutionary pathway and cryptic features of plant populations and such information can be used to select the best breeding strategy. This review also highlights the importance of proteomic analysis as a breeding tool. In this regard, protein diversification, as well as the impact of normal and stress-responsive proteins on morphometric and physiological behaviors, could be used in breeding programs. These findings have immense potential for improving plant production and, therefore, can be regarded as prospective breeding platforms for medicinal plants that have an autogamous mode of reproduction. Finally, this review suggests that novel site-directed genome editing approaches such as TALENs (Transcription Activator-Like Effector Nucleases) and CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-

associated protein-9 nuclease) systems together with other new plant breeding technologies (NPBT) should simultaneously be taken into consideration for improvement of pharmaceutical plants.

Varshney, G. K., B. Carrington, et al. "A high-throughput functional genomics workflow based on CRISPR/Cas9-mediated targeted mutagenesis in zebrafish." *Nat Protoc.* 2016 Dec;11(12):2357-2375. doi: [10.1038/nprot.2016.141](https://doi.org/10.1038/nprot.2016.141). Epub 2016 Oct 27.

The zebrafish is a popular model organism for studying development and disease, and genetically modified zebrafish provide an essential tool for functional genomic studies. Numerous publications have demonstrated the efficacy of gene targeting in zebrafish using CRISPR/Cas9, and they have included descriptions of a variety of tools and methods for guide RNA synthesis and mutant identification. However, most of the published techniques are not readily scalable to increase throughput. We recently described a CRISPR/Cas9-based high-throughput mutagenesis and phenotyping pipeline in zebrafish. Here, we present a complete workflow for this pipeline, including target selection; cloning-free single-guide RNA (sgRNA) synthesis; microinjection; validation of the target-specific activity of the sgRNAs; founder screening to identify germline-transmitting mutations by fluorescence PCR; determination of the exact lesion by Sanger or next-generation sequencing (including software for analysis); and genotyping in the F1 or subsequent generations. Using these methods, sgRNAs can be evaluated in 3 d, zebrafish germline-transmitting mutations can be identified within 3 months and stable lines can be established within 6 months. Realistically, two researchers can target tens to hundreds of genes per year using this protocol.

Vercruyse, T., J. De Bie, et al. "The second-generation exportin-1 inhibitor KPT-8602 demonstrates potent activity against acute lymphoblastic leukemia." *Clin Cancer Res.* 2016 Oct 25. pii: [clinres.1580.2016](https://doi.org/10.1158/1538-7443.CCR16016).

PURPOSE: Human exportin-1 (XPO1) is the key nuclear-cytoplasmic transport protein that exports many cargo proteins out of the nucleus. Inducing nuclear accumulation of these proteins by inhibition of XPO1 causes cancer cell death. First clinical validation of pharmacological inhibition of XPO1 was obtained with the Selective Inhibitor of Nuclear Export (SINE) compound selinexor (KPT-330) demonstrating activity in Phase-II/IIb clinical trials when dosed 1 - 3 times weekly. The second-generation SINE compound KPT-8602 shows improved tolerability and can be dosed daily. Here we investigate and validate the drug-target interaction of KPT-8602 and explore its activity against acute lymphoblastic leukemia (ALL).

EXPERIMENTAL DESIGN: We examined the effect of KPT-8602 on XPO1 function and XPO1-cargo as well as on a panel of leukemia cell lines. Mutant XPO1 leukemia cells were designed to validate KPT-8602's drug-target interaction. In vivo, anti-ALL activity was measured in a mouse ALL model and patient-derived ALL xenograft models. **RESULTS:** KPT-8602 induced caspase-dependent apoptosis in a panel of leukemic cell lines in vitro. Using CRISPR/Cas9 genome editing we demonstrated the specificity of KPT-8602 for cysteine 528 in the cargo-binding groove of XPO1 and validated the drug target interaction. In vivo, KPT-8602 showed potent anti-leukemia activity in a mouse ALL model as well as in patient-derived T- and B-ALL xenograft models without affecting normal hematopoiesis. **CONCLUSIONS:** KPT-8602 is highly specific for XPO1 inhibition and demonstrates potent anti-leukemic activity supporting clinical application of the second-generation SINE compound for the treatment of ALL.

Verissimo, C. S., R. M. Overmeer, et al. "Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening." *Elife.* 2016 Nov 15;5. pii: [e18489](https://doi.org/10.7554/eLife.18489). doi: [10.7554/eLife.18489](https://doi.org/10.7554/eLife.18489).

Colorectal cancer (CRC) organoids can be derived from almost all CRC patients and therefore capture the genetic diversity of this disease. We assembled a panel of CRC organoids carrying either wild-type or mutant RAS, as well as normal organoids and tumor organoids with a CRISPR-introduced oncogenic KRAS mutation. Using this panel, we evaluated RAS pathway inhibitors and drug combinations that are currently in clinical trial for RAS mutant cancers. Presence of mutant RAS correlated strongly with resistance to these targeted therapies. This was observed in tumorigenic as well as in normal organoids. Moreover, dual inhibition of the EGFR-MEK-ERK pathway in RAS mutant organoids induced a transient cell-cycle arrest rather than cell death. In vivo drug response of xenotransplanted RAS mutant organoids confirmed this growth arrest upon pan-HER/MEK combination therapy. Altogether, our studies demonstrate the potential of patient-derived CRC organoid libraries in evaluating inhibitors and drug combinations in a preclinical setting.

Vijai, J., S. Topka, et al. "A Recurrent ERCC3 Truncating Mutation Confers Moderate Risk for Breast Cancer." *Cancer Discov.* 2016 Nov;6(11):1267-1275. Epub 2016 Sep 21.

Known gene mutations account for approximately 50% of the hereditary risk for breast cancer. Moderate and low penetrance variants, discovered by genomic approaches, account for an as-

yet-unknown proportion of the remaining heritability. A truncating mutation c.325C>T:p.Arg109* (R109X) in the ATP-dependent helicase ERCC3 was observed recurrently among exomes sequenced in BRCA wild-type, breast cancer-affected individuals of Ashkenazi Jewish ancestry. Modeling of the mutation in ERCC3-deficient or CRISPR/Cas9-edited cell lines showed a consistent pattern of reduced expression of the protein and concomitant hypomorphic functionality when challenged with UVC exposure or treatment with the DNA alkylating agent IlludinS. Overexpressing the mutant protein in ERCC3-deficient cells only partially rescued their DNA repair-deficient phenotype. Comparison of frequency of this recurrent mutation in over 6,500 chromosomes of breast cancer cases and 6,800 Ashkenazi controls showed significant association with breast cancer risk (ORBC = 1.53, OREC+ = 1.73), particularly for the estrogen receptor-positive subset ($P < 0.007$). SIGNIFICANCE: A functionally significant recurrent ERCC3 mutation increased the risk for breast cancer in a genetic isolate. Mutated cell lines showed lower survival after in vitro exposure to DNA-damaging agents. Thus, similar to tumors arising in the background of homologous repair defects, mutations in nucleotide excision repair genes such as ERCC3 could constitute potential therapeutic targets in a subset of hereditary breast cancers. Cancer Discov; 6(11); 1267-75. (c)2016 AACR. This article is highlighted in the In This Issue feature, p. 1197.

Waaaijers, S., J. Munoz, et al. "A tissue-specific protein purification approach in *Caenorhabditis elegans* identifies novel interaction partners of DLG-1/Discs large." *BMC Biol.* 2016 Aug 9;14:66. doi: [10.1186/s12915-016-0286-x](https://doi.org/10.1186/s12915-016-0286-x).

BACKGROUND: Affinity purification followed by mass spectrometry (AP/MS) is a widely used approach to identify protein interactions and complexes. In multicellular organisms, the accurate identification of protein complexes by AP/MS is complicated by the potential heterogeneity of complexes in different tissues. Here, we present an in vivo biotinylation-based approach for the tissue-specific purification of protein complexes from *Caenorhabditis elegans*. Tissue-specific biotinylation is achieved by the expression in select tissues of the bacterial biotin ligase BirA, which biotinylates proteins tagged with the Avi peptide. **RESULTS:** We generated N- and C-terminal tags combining GFP with the Avi peptide sequence, as well as four BirA driver lines expressing BirA ubiquitously and specifically in the seam and hyp7 epidermal cells, intestine, or neurons. We validated the ability of our approach to identify bona fide protein interactions by identifying the known LGL-1 interaction partners PAR-6 and PKC-3. Purification of the Discs large protein DLG-1

identified several candidate interaction partners, including the AAA-type ATPase ATAD-3 and the uncharacterized protein MAPH-1.1. We have identified the domains that mediate the DLG-1/ATAD-3 interaction, and show that this interaction contributes to *C. elegans* development. MAPH-1.1 co-purified specifically with DLG-1 purified from neurons, and shared limited homology with the microtubule-associated protein MAP1A, a known neuronal interaction partner of mammalian DLG4/PSD95. A CRISPR/Cas9-engineered GFP::MAPH-1.1 fusion was broadly expressed and co-localized with microtubules. **CONCLUSIONS:** The method we present here is able to purify protein complexes from specific tissues. We uncovered a series of DLG-1 interactors, and conclude that ATAD-3 is a biologically relevant interaction partner of DLG-1. Finally, we conclude that MAPH-1.1 is a microtubule-associated protein of the MAP1 family and a candidate neuron-specific interaction partner of DLG-1.

Wakabayashi, S., N. Sawamura, et al. "Ohgata, the Single Drosophila Ortholog of Human Cereblon, Regulates Insulin Signaling-dependent Organismic Growth." *J Biol Chem.* 2016 Nov 25;291(48):25120-25132. Epub 2016 Oct 4.

Cereblon (CRBN) is a substrate receptor of the E3 ubiquitin ligase complex that is highly conserved in animals and plants. CRBN proteins have been implicated in various biological processes such as development, metabolism, learning, and memory formation, and their impairment has been linked to autosomal recessive non-syndromic intellectual disability and cancer. Furthermore, human CRBN was identified as the primary target of thalidomide teratogenicity. Data on functional analysis of CRBN family members in vivo, however, are still scarce. Here we identify Ohgata (OHGT), the *Drosophila* ortholog of CRBN, as a regulator of insulin signaling-mediated growth. Using ohgt mutants that we generated by targeted mutagenesis, we show that its loss results in increased body weight and organ size without changes of the body proportions. We demonstrate that ohgt knockdown in the fat body, an organ analogous to mammalian liver and adipose tissue, phenocopies the growth phenotypes. We further show that overgrowth is due to an elevation of insulin signaling in ohgt mutants and to the down-regulation of inhibitory cofactors of circulating *Drosophila* insulin-like peptides (DILPs), named acid-labile subunit and imaginal morphogenesis protein-late 2. The two inhibitory proteins were previously shown to be components of a heterotrimeric complex with growth-promoting DILP2 and DILP5. Our study reveals OHGT as a novel regulator of insulin-dependent organismic growth in *Drosophila*.

Walton, J., J. Blagih, et al. "CRISPR/Cas9-Mediated Trp53 and Brca2 Knockout to Generate Improved Murine Models of Ovarian High-Grade Serous Carcinoma." *Cancer Res.* 2016 Oct 15;76(20):6118-6129. Epub 2016 Aug 16.

There is a need for transplantable murine models of ovarian high-grade serous carcinoma (HGSC) with regard to mutations in the human disease to assist investigations of the relationships between tumor genotype, chemotherapy response, and immune microenvironment. In addressing this need, we performed whole-exome sequencing of ID8, the most widely used transplantable model of ovarian cancer, covering 194,000 exomes at a mean depth of 400x with 90% exons sequenced >50x. We found no functional mutations in genes characteristic of HGSC (Trp53, Brca1, Brca2, Nfl, and Rb1), and p53 remained transcriptionally active. Homologous recombination in ID8 remained intact in functional assays. Further, we found no mutations typical of clear cell carcinoma (Arid1a, Pik3ca), low-grade serous carcinoma (Braf), endometrioid (Ctnnb1), or mucinous (Kras) carcinomas. Using CRISPR/Cas9 gene editing, we modeled HGSC by generating novel ID8 derivatives that harbored single (Trp53^{-/-}) or double (Trp53^{-/-};Brca2^{-/-}) suppressor gene deletions. In these mutants, loss of p53 alone was sufficient to increase the growth rate of orthotopic tumors with significant effects observed on the immune microenvironment. Specifically, p53 loss increased expression of the myeloid attractant CCL2 and promoted the infiltration of immunosuppressive myeloid cell populations into primary tumors and their ascites. In Trp53^{-/-};Brca2^{-/-} mutant cells, we documented a relative increase in sensitivity to the PARP inhibitor rucaparib and slower orthotopic tumor growth compared with Trp53^{-/-} cells, with an appearance of intratumoral tertiary lymphoid structures rich in CD3⁺ T cells. This work validates new CRISPR-generated models of HGSC to investigate its biology and promote mechanism-based therapeutics discovery. *Cancer Res*; 76(20); 6118-29. (c)2016 AACR.

Wang, H. and W. Sun "CRISPR-mediated targeting of HER2 inhibits cell proliferation through a dominant negative mutation." *Cancer Lett.* 2017 Jan 28;385:137-143. doi: 10.1016/j.canlet.2016.10.033. Epub 2016 Nov 1.

With the discovery of the CRISPR/Cas9 technology, genome editing could be performed in a rapid, precise and effective manner. Its potential applications in functional interrogation of cancer-causing genes and cancer therapy have been extensively explored. In this study, we demonstrated the use of the CRISPR/Cas9 system to directly target

the oncogene HER2. Directing Cas9 to exons of the HER2 gene inhibited cell growth in breast cancer cell lines that harbor amplification of the HER2 locus. The inhibitory effect was potentiated with the addition of PARP inhibitors. Unexpectedly, CRISPR-induced mutations did not significantly affect the level of HER2 protein expression. Instead, CRISPR targeting appeared to exert its effect through a dominant negative mutation. This HER2 mutant interfered with the MAPK/ERK axis of HER2 downstream signaling. Our work provides a novel mechanism underlying the anti-cancer effects of HER2-targeting by CRISPR/Cas9, which is distinct from the clinical drug Herceptin. In addition, it opens up the possibility that incomplete CRISPR targeting of certain oncogenes could still have therapeutic value by generation of dominant negative mutants.

Wang, J., X. Song, et al. "Establishment of MAGEC2-knockout cells and functional investigation of MAGEC2 in tumor cells." *Cancer Sci.* 2016 Dec;107(12):1888-1897. doi: 10.1111/cas.13082. Epub 2016 Nov 25.

Cancer/testis antigen MAGEC2, a member of the type I melanoma-associated antigen family, is expressed in a wide variety of cancer types but not in normal somatic cells. MAGEC2 has long been recognized as a tumor-specific target, however, its functions remain largely unknown. In this study, we established MAGEC2-knockout A375 melanoma cell lines using the CRISPR/Cas9 system. Seven clonal cell lines were generated by using four single guide RNAs targeting the coding region of the MAGEC2 gene, which produced indels that abolished MAGEC2 protein expression. To identify the differentially expressed protein profiles associated with MAGEC2 loss, isobaric tag for relative quantitation-based comparative proteomics experiments were carried out on the MAGEC2-knockout and control A375 cells. Mining of the proteomics data identified a total 224 (61.6% upregulated and 38.4% downregulated) proteins to be significantly altered in expression level in MAGEC2-knockout cells. Ingenuity Pathway Analysis indicated that the significantly altered proteins were involved in critical neoplasia-related biological functions such as cell death, proliferation, and movement. Gene ontology analysis identified "apoptosis signaling" as the top-most upregulated pathway associated with MAGEC2 loss. We showed that knockout or knockdown of the MAGEC2 gene sensitized melanoma cells to tumor necrosis factor- α -induced apoptosis. Interestingly, actin-based motility by Rho and RhoA signaling, known to promote cell migration, were also identified as the top downregulated pathways in MAGEC2-knockout A375 cells. In short, our study provides a suitable cell model

for exploring the biological functions of MAGEC2 in malignant cells, and sheds light on the molecular pathway by which MAGEC2 promotes tumor development.

Wang, L., H. Wang, et al. "New strategies for targeting drug combinations to overcome mutation-driven drug resistance." *Semin Cancer Biol.* 2016 Nov 10. pii: S1044-579X(16)30071-2. doi: [10.1016/j.semcancer.2016.11.002](https://doi.org/10.1016/j.semcancer.2016.11.002).

Targeted therapies are suggested as an effective alternative for patients with cancer that harbor mutations, but treatment outcomes are frequently limited by primary or acquired drug resistance. The present review describes potential mechanisms of primary or acquired drug resistances to provide a resource for considering how to be overcome. We focus on strategies of targeted drug combinations to minimize the development of drug resistance within the context how resistance develops. Strategies benefit from the combined use of "omics" technologies, i.e., high-throughput functional genomics data, pharmacogenomics, or genome-wide CRISPR-Cas9 screening, to analyze and design targeted drug combinations for mutation-driven drug resistance. We also introduce new insights towards pathway-centric combined therapies as an alternative to overcome the heterogeneity and benefit patient prognoses.

Wang, Y., Y. C. Koay, et al. "Redefining the phenotype of Heat shock protein 90 (Hsp90) inhibitors." *Chemistry.* 2016 Nov 8. doi: [10.1002/chem.201604807](https://doi.org/10.1002/chem.201604807).

Comparing the phenotypes produced when cells are treated with the Hsp90 inhibitors AUY922 or 17-AAG (classical inhibitors) to cells with Hsp90alpha knocked down reveals that the knockdown phenotype is unique from that produced by the inhibitors. Pull-down assays using classical inhibitors verify that these molecules do not bind to Hsp90 as their primary target. Classical inhibitors induce similar protein markers as other anti-cancer therapies Cisplatin and bortezomib, suggesting that AUY922 and 17-AAG act on targets other than Hsp90 and kill cells through multiple mechanisms. Comparing these classical inhibitors to C-terminal Hsp90 modulators reveals that C-terminal modulators effectively bind to Hsp90, and induce phenotypic markers consistent with the CRISPR knockdown data. Our findings challenge the current interpretation of Hsp90 inhibitors suggest that a large body of literature describing the Hsp90 phenotype and inhibitors requires re-examination.

Wei, Y., K. Li, et al. "Loss of ZNF32 augments the regeneration of nervous lateral line system through negative regulation of SOX2 transcription."

Oncotarget. 2016 Oct 25;7(43):70420-70436. doi: [10.18632/oncotarget.11895](https://doi.org/10.18632/oncotarget.11895).

Human zinc finger protein 32 (ZNF32) is a Cys2-His2 zinc-finger transcription factor that plays an important role in cell fate, yet much of its function remains unknown. Here, we reveal that the zebrafish ZNF32 homologue zfZNF32 is expressed in the nervous system, particularly in the lateral line system. ZfZNF32 knock-out zebrafish (zfZNF^{-/-}) were generated using the CRISPR-associated protein 9 system. We found that the regenerative capacity of the lateral line system was increased in zfZNF^{-/-} upon hair cell damage compared with the wild type. Moreover, SOX2 was essential for the zfZNF32-dependent modulation of lateral line system regeneration. Mechanistic studies showed that ZNF32 suppressed SOX2 transcription by directly binding to a consensus sequence (5'-gcattt-3') in the SOX2 promoter. In addition, ZNF32 localizes to the nucleus, and we have identified that amino acids 1-169 (Aa 1-169) and each of three independent nuclear localization signals (NLSs) in ZNF32 are indispensable for ZNF32 nuclear trafficking. Mutating the NLSs disrupted the inhibitory effect of ZNF32 in SOX2 expression, highlighting the critical role of the NLSs in ZNF32 function. Our findings reveal a pivotal role for ZNF32 function in SOX2 expression and regeneration regulation.

Wen, K., L. Yang, et al. "Critical roles of long noncoding RNAs in Drosophila spermatogenesis." *Genome Res.* 2016 Sep;26(9):1233-44. doi: [10.1101/gr.199547.115](https://doi.org/10.1101/gr.199547.115). Epub 2016 Aug 11.

Long noncoding RNAs (lncRNAs), a recently discovered class of cellular RNAs, play important roles in the regulation of many cellular developmental processes. Although lncRNAs have been systematically identified in various systems, most of them have not been functionally characterized in vivo in animal models. In this study, we identified 128 testis-specific Drosophila lncRNAs and knocked out 105 of them using an optimized three-component CRISPR/Cas9 system. Among the lncRNA knockouts, 33 (31%) exhibited a partial or complete loss of male fertility, accompanied by visual developmental defects in late spermatogenesis. In addition, six knockouts were fully or partially rescued by transgenes in a trans configuration, indicating that those lncRNAs primarily work in trans. Furthermore, gene expression profiles for five lncRNA mutants revealed that testis-specific lncRNAs regulate global gene expression, orchestrating late male germ cell differentiation. Compared with coding genes, the testis-specific lncRNAs evolved much faster. Moreover, lncRNAs of greater functional importance exhibited higher sequence conservation, suggesting that they are under constant evolutionary selection. Collectively, our

results reveal critical functions of rapidly evolving testis-specific lncRNAs in late *Drosophila* spermatogenesis.

Williams, C. A., R. Fernandez-Alonso, et al. "Erk5 Is a Key Regulator of Naive-Primed Transition and Embryonic Stem Cell Identity." Cell Rep. 2016 Aug 16;16(7):1820-8. doi: 10.1016/j.celrep.2016.07.033. Epub 2016 Aug 4.

Embryonic stem cells (ESCs) can self-renew or differentiate into any cell type, a phenomenon known as pluripotency. Distinct pluripotent states, termed naive and primed pluripotency, have been described. However, the mechanisms that control naive-primed pluripotent transition are poorly understood. Here, we perform a targeted screen for kinase inhibitors, which modulate the naive-primed pluripotent transition. We find that XMD compounds, which selectively inhibit Erk5 kinase and BET bromodomain family proteins, drive ESCs toward primed pluripotency. Using compound selectivity engineering and CRISPR/Cas9 genome editing, we reveal distinct functions for Erk5 and Brd4 in pluripotency regulation. We show that Erk5 signaling maintains ESCs in the naive state and suppresses progression toward primed pluripotency and neuroectoderm differentiation. Additionally, we identify a specialized role for Erk5 in defining ESC lineage selection, whereby Erk5 inhibits a cardiomyocyte-specific differentiation program. Our data therefore reveal multiple critical functions for Erk5 in controlling ESC identity.

Wu, J., L. Li, et al. "Translesion synthesis of O4-alkylthymidine lesions in human cells." Nucleic Acids Res. 2016 Nov 2;44(19):9256-9265. Epub 2016 Jul 27.

Environmental exposure, endogenous metabolism and cancer chemotherapy can give rise to alkylation of DNA, and the resulting alkylated thymidine (alkyldT) lesions were found to be poorly repaired and persistent in mammalian tissues. Unrepaired DNA lesions may compromise genomic integrity by inhibiting DNA replication and inducing mutations in these processes. In this study, we explored how eight O4-alkyldT lesions, with the alkyl group being a Me, Et, nPr, iPr, nBu, iBu, (R)-sBu and (S)-sBu, are recognized by DNA replication machinery in HEK293T human embryonic kidney cells. We found that the O4-alkyldT lesions are moderately blocking to DNA replication, with the bypass efficiencies ranging from 20 to 33% in HEK293T cells, and these lesions induced substantial frequencies T->C transition mutation. We also conducted the replication experiments in the isogenic cells where individual translesion synthesis (TLS) DNA polymerases were depleted by the CRISPR/Cas9

genome editing method. Our results showed that deficiency in Pol eta or Pol zeta, but not Pol kappa or Pol iota, led to pronounced drops in bypass efficiencies for all the O4-alkyldT lesions except O4-MedT. In addition, depletion of Pol zeta resulted in significant decreases in T->C mutation frequencies for all the O4-alkyldT lesions except O4-MedT and O4-nBudT. Thus, our study provided important new knowledge about the cytotoxic and mutagenic properties of the O4-alkyldT lesions and defined the roles of TLS polymerases in bypassing these lesions in human cells.

Xia, P., X. Zhang, et al. "Functional Toxicogenomic Assessment of Triclosan in Human HepG2 Cells Using Genome-Wide CRISPR-Cas9 Screening." Environ Sci Technol. 2016 Oct 4;50(19):10682-10692. Epub 2016 Aug 13.

There are thousands of chemicals used by humans and detected in the environment for which limited or no toxicological data are available. Rapid and cost-effective approaches for assessing the toxicological properties of chemicals are needed. We used CRISPR-Cas9 functional genomic screening to identify the potential molecular mechanism of a widely used antimicrobial triclosan (TCS) in HepG2 cells. Resistant genes at IC50 (the concentration causing a 50% reduction in cell viability) were significantly enriched in the adherens junction pathway, MAPK signaling pathway, and PPAR signaling pathway, suggesting a potential role in the molecular mechanism of TCS-induced cytotoxicity. Evaluation of the top-ranked resistant genes, FTO (encoding an mRNA demethylase) and MAP2K3 (a MAP kinase kinase family gene), revealed that their loss conferred resistance to TCS. In contrast, sensitive genes at IC10 and IC20 were specifically enriched in pathways involved with immune responses, which was concordant with transcriptomic profiling of TCS at concentrations of <IC10. It is suggested that the CRISPR-Cas9 fingerprint may reveal the patterns of TCS toxicity at low concentration levels. Moreover, we retrieved the potential connection between CRISPR-Cas9 fingerprint and disease terms, obesity, and breast cancer from an existing chemical-gene-disease database. Overall, CRISPR-Cas9 functional genomic screening offers an alternative approach for chemical toxicity testing.

Xie, H., C. Peng, et al. "Chronic Myelogenous Leukemia- Initiating Cells Require Polycomb Group Protein EZH2." Cancer Discov. 2016 Nov;6(11):1237-1247. Epub 2016 Sep 14.

Tyrosine kinase inhibitors (TKI) have revolutionized chronic myelogenous leukemia (CML) management. Disease eradication, however, is

hampered by innate resistance of leukemia-initiating cells (LIC) to TKI-induced killing, which also provides the basis for subsequent emergence of TKI-resistant mutants. We report that EZH2, the catalytic subunit of Polycomb Repressive Complex 2 (PRC2), is overexpressed in CML LICs and required for colony formation and survival and cell-cycle progression of CML cell lines. A critical role for EZH2 is supported by genetic studies in a mouse CML model. Inactivation of Ezh2 in conventional conditional mice and through CRISPR/Cas9-mediated gene editing prevents initiation and maintenance of disease and survival of LICs, irrespective of BCR-ABL1 mutational status, and extends survival. Expression of the EZH2 homolog EZH1 is reduced in EZH2-deficient CML LICs, creating a scenario resembling complete loss of PRC2. EZH2 dependence of CML LICs raises prospects for improved therapy of TKI-resistant CML and/or eradication of disease by addition of EZH2 inhibitors. **SIGNIFICANCE:** This work defines EZH2 as a selective vulnerability for CML cells and their LICs, regardless of BCR-ABL1 mutational status. Our findings provide an experimental rationale for improving disease eradication through judicious use of EZH2 inhibitors within the context of standard-of-care TKI therapy. *Cancer Discov*; 6(11); 1237-47. (c)2016 AACR. See related article by Scott et al., p. 1248 This article is highlighted in the In This Issue feature, p. 1197.

Xu, C., X. Qi, et al. "piggyBac mediates efficient in vivo CRISPR library screening for tumorigenesis in mice." *Proc Natl Acad Sci U S A*. 2017 Jan 6. pii: [201615735](https://doi.org/10.1073/pnas.1615735114). doi: [10.1073/pnas.1615735114](https://doi.org/10.1073/pnas.1615735114).

CRISPR/Cas9 is becoming an increasingly important tool to functionally annotate genomes. However, because genome-wide CRISPR libraries are mostly constructed in lentiviral vectors, in vivo applications are severely limited as a result of difficulties in delivery. Here, we examined the piggyBac (PB) transposon as an alternative vehicle to deliver a guide RNA (gRNA) library for in vivo screening. Although tumor induction has previously been achieved in mice by targeting cancer genes with the CRISPR/Cas9 system, in vivo genome-scale screening has not been reported. With our PB-CRISPR libraries, we conducted an in vivo genome-wide screen in mice and identified genes mediating liver tumorigenesis, including known and unknown tumor suppressor genes (TSGs). Our results demonstrate that PB can be a simple and nonviral choice for efficient in vivo delivery of CRISPR libraries.

Yang, H., P. Gao, et al. "PAM-Dependent Target DNA Recognition and Cleavage by C2c1 CRISPR-Cas Endonuclease." *Cell*. 2016 Dec 15;167(7):1814-

1828.e12. doi: [10.1016/j.cell.2016.11.053](https://doi.org/10.1016/j.cell.2016.11.053).

C2c1 is a newly identified guide RNA-mediated type V-B CRISPR-Cas endonuclease that site-specifically targets and cleaves both strands of target DNA. We have determined crystal structures of *Alicyclobacillus acidoterrestris* C2c1 (AacC2c1) bound to sgRNA as a binary complex and to target DNAs as ternary complexes, thereby capturing catalytically competent conformations of AacC2c1 with both target and non-target DNA strands independently positioned within a single RuvC catalytic pocket. Moreover, C2c1-mediated cleavage results in a staggered seven-nucleotide break of target DNA. crRNA adopts a pre-ordered five-nucleotide A-form seed sequence in the binary complex, with release of an inserted tryptophan, facilitating zippering up of 20-bp guide RNA:target DNA heteroduplex on ternary complex formation. Notably, the PAM-interacting cleft adopts a "locked" conformation on ternary complex formation. Structural comparison of C2c1 ternary complexes with their Cas9 and Cpf1 counterparts highlights the diverse mechanisms adopted by these distinct CRISPR-Cas systems, thereby broadening and enhancing their applicability as genome editing tools.

Yang, Y., J. G. Qiu, et al. "Targeting ABCB1-mediated tumor multidrug resistance by CRISPR/Cas9-based genome editing." *Am J Transl Res*. 2016 Sep 15;8(9):3986-3994. eCollection 2016.

The RNA-guided clustered regularly interspaced short palindromic (CRISPR) in combination with a CRISPR-associated nuclease 9 (Cas9) nuclease system is a new rapid and precise technology for genome editing. In the present study, we applied the CRISPR/Cas9 system to target ABCB1 (also named MDR1) gene which encodes a 170 kDa transmembrane glycoprotein (P-glycoprotein/P-gp) transporting multiple types of chemotherapeutic drugs including taxanes, epipodophyllotoxins, vinca alkaloids and anthracyclines out of cells to contribute multidrug resistance (MDR) in cancer cells. Our data showed that knockout of ABCB1 by CRISPR/Cas9 system was successfully achieved with two target sgRNAs in two MDR cancer cells due to the alteration of genome sequences. Knockout of ABCB1 by CRISPR/Cas9 system significantly enhances the sensitivity of ABCB1 substrate chemotherapeutic agents and the intracellular accumulation of rhodamine 123 and doxorubicin in MDR cancer cells. Although now there are lots of limitations to the application of CRISPR/Cas9 for editing cancer genes in human patients, our study provides valuable clues for the use of the CRISPR/Cas9 technology in the investigation and conquest of cancer MDR.

Yang, Z., C. Li, et al. "Single-cell Sequencing Reveals Variants in ARID1A, GPRC5A and MLL2 Driving Self-renewal of Human Bladder Cancer Stem Cells." *Eur Urol.* 2017 Jan;71(1):8-12. doi: [10.1016/j.eururo.2016.06.025](https://doi.org/10.1016/j.eururo.2016.06.025). Epub 2016 Jul 4.

Cancer stem cells are considered responsible for many important aspects of tumors such as their self-renewal, tumor-initiating, drug-resistance and metastasis. However, the genetic basis and origination of human bladder cancer stem cells (BCSCs) remains unknown. Here, we conducted single-cell sequencing on 59 cells including BCSCs, bladder cancer non-stem cells (BCNSCs), bladder epithelial stem cells (BESCs) and bladder epithelial non-stem cells (BENSCs) from three bladder cancer (BC) specimens. Specifically, BCSCs demonstrate clonal homogeneity and suggest their origin from BESCs or BCNSCs through phylogenetic analysis. Moreover, 21 key altered genes were identified in BCSCs including six genes not previously described in BC (ETS1, GPRC5A, MKL1, PAWR, PITX2 and RGS9BP). Co-mutations of ARID1A, GPRC5A and MLL2 introduced by CRISPR/Cas9 significantly enhance the capabilities of self-renewal and tumor-initiating of BCNSCs. To our knowledge, our study first provides an overview of the genetic basis of human BCSCs with single-cell sequencing and demonstrates the biclonal origin of human BCSCs via evolution analysis. **PATIENT SUMMARY:** Human bladder cancer stem cells show the high level of consistency and may derived from bladder epithelial stem cells or bladder cancer non-stem cells. Mutations of ARID1A, GPRC5A and MLL2 grant bladder cancer non-stem cells the capability of self-renewal.

Yasuda, H., C. D. Oh, et al. "A Novel Regulatory Mechanism of Type II Collagen Expression via a SOX9-dependent Enhancer in Intron 6." *J Biol Chem.* 2017 Jan 13;292(2):528-538. doi: [10.1074/jbc.M116.758425](https://doi.org/10.1074/jbc.M116.758425). Epub 2016 Nov 23.

Type II collagen alpha1 is specific for cartilaginous tissues, and mutations in its gene are associated with skeletal diseases. Its expression has been shown to be dependent on SOX9, a master transcription factor required for chondrogenesis that binds to an enhancer region in intron 1. However, CHIP sequencing revealed that SOX9 does not strongly bind to intron 1, but rather it binds to intron 6 and a site 30 kb upstream of the transcription start site. Here, we aimed to determine the role of the novel SOX9-binding site in intron 6. We prepared reporter constructs that contain a Col2a1 promoter, intron 1 with or without intron 6, and the luciferase gene. Although the reporter constructs were not activated by SOX9 alone, the construct that contained both introns 1 and 6 was activated 5-10-fold by the SOX9/SOX5 or

the SOX9/SOX6 combination in transient-transfection assays in 293T cells. This enhancement was also observed in rat chondrosarcoma cells that stably expressed the construct. CRISPR/Cas9-induced deletion of intron 6 in RCS cells revealed that a 10-bp region of intron 6 is necessary both for Col2a1 expression and SOX9 binding. Furthermore, SOX9, but not SOX5, binds to this region as demonstrated in an electrophoretic mobility shift assay, although both SOX9 and SOX5 bind to a larger 325-bp fragment of intron 6 containing this small sequence. These findings suggest a novel mechanism of action of SOX5/6; namely, the SOX9/5/6 combination enhances Col2a1 transcription through a novel enhancer in intron 6 together with the enhancer in intron 1.

Ye, H., X. Zhang, et al. "Ranking novel cancer driving synthetic lethal gene pairs using TCGA data." *Oncotarget.* 2016 Aug 23;7(34):55352-55367. doi: [10.18632/oncotarget.10536](https://doi.org/10.18632/oncotarget.10536).

Synthetic lethality (SL) has emerged as a promising approach to cancer therapy. In contrast to the costly and labour-intensive genome-wide siRNA or CRISPR-based human cell line screening approaches, computational approaches to prioritize potential synthetic lethality pairs for further experimental validation represent an attractive alternative. In this study, we propose an efficient and comprehensive in-silico pipeline to rank novel SL gene pairs by mining vast amounts of accumulated tumor high-throughput sequencing data in The Cancer Genome Atlas (TCGA), coupled with other protein interaction networks and cell line information. Our pipeline integrates three significant features, including mutation coverage in TCGA, driver mutation probability and the quantified cancer network information centrality, into a ranking model for SL gene pair identification, which is presented as the first learning-based method for SL identification. As a result, 107 potential SL gene pairs were obtained from the top 10 results covering 11 cancers. Functional analysis of these genes indicated that several promising pathways were identified, including the DNA repair related Fanconi Anemia pathway and HIF-1 signaling pathway. In addition, 4 SL pairs, mTOR-TP53, VEGFR2-TP53, EGFR-TP53, ATM-PRKCA, were validated using drug sensitivity information in the cancer cell line databases CCLE or NCI60. Interestingly, significant differences in the cell growth of mTOR siRNA or EGFR siRNA knock-down were detected between cancer cells with wild type TP53 and mutant TP53. Our study indicates that the pre-screening of potential SL gene pairs based on the large genomics data repertoire of tumor tissues and cancer cell lines could substantially expedite the identification of synthetic lethal gene pairs for cancer therapy.

Ye, J., Y. Gu, et al. "IDH1 deficiency attenuates gluconeogenesis in mouse liver by impairing amino acid utilization." *Proc Natl Acad Sci U S A*. 2017 Jan 10;114(2):292-297. doi: 10.1073/pnas.1618605114. Epub 2016 Dec 23.

Although the enzymatic activity of isocitrate dehydrogenase 1 (IDH1) was defined decades ago, its functions in vivo are not yet fully understood. Cytosolic IDH1 converts isocitrate to alpha-ketoglutarate (alpha-KG), a key metabolite regulating nitrogen homeostasis in catabolic pathways. It was thought that IDH1 might enhance lipid biosynthesis in liver or adipose tissue by generating NADPH, but we show here that lipid contents are relatively unchanged in both IDH1-null mouse liver and IDH1-deficient HepG2 cells generated using the CRISPR-Cas9 system. Instead, we found that IDH1 is critical for liver amino acid (AA) utilization. Body weights of IDH1-null mice fed a high-protein diet (HPD) were abnormally low. After prolonged fasting, IDH1-null mice exhibited decreased blood glucose but elevated blood alanine and glycine compared with wild-type (WT) controls. Similarly, in IDH1-deficient HepG2 cells, glucose consumption was increased, but alanine utilization and levels of intracellular alpha-KG and glutamate were reduced. In IDH1-deficient primary hepatocytes, gluconeogenesis as well as production of ammonia and urea were decreased. In IDH1-deficient whole livers, expression levels of genes involved in AA metabolism were reduced, whereas those involved in gluconeogenesis were up-regulated. Thus, IDH1 is critical for AA utilization in vivo and its deficiency attenuates gluconeogenesis primarily by impairing alpha-KG-dependent transamination of glucogenic AAs such as alanine.

Yi, L. and J. Li "CRISPR-Cas9 therapeutics in cancer: promising strategies and present challenges." *Biochim Biophys Acta*. 2016 Dec;1866(2):197-207. doi: 10.1016/j.bbcan.2016.09.002. Epub 2016 Sep 15.

Cancer is characterized by multiple genetic and epigenetic alterations that drive malignant cell proliferation and confer chemoresistance. The ability to correct or ablate such mutations holds immense promise for combating cancer. Recently, because of its high efficiency and accuracy, the CRISPR-Cas9 genome editing technique has been widely used in cancer therapeutic explorations. Several studies used CRISPR-Cas9 to directly target cancer cell genomic DNA in cellular and animal cancer models which have shown therapeutic potential in expanding our anticancer protocols. Moreover, CRISPR-Cas9 can also be employed to fight oncogenic infections, explore anticancer drugs, and engineer immune cells and oncolytic viruses for cancer immunotherapeutic

applications. Here, we summarize these preclinical CRISPR-Cas9-based therapeutic strategies against cancer, and discuss the challenges and improvements in translating therapeutic CRISPR-Cas9 into clinical use, which will facilitate better application of this technique in cancer research. Further, we propose potential directions of the CRISPR-Cas9 system in cancer therapy.

Yin, Y., J. Zhong, et al. "TRIM11, a direct target of miR-24-3p, promotes cell proliferation and inhibits apoptosis in colon cancer." *Oncotarget*. 2016 Dec 27;7(52):86755-86765. doi: 10.18632/oncotarget.13550.

TRIM11 (tripartite motif-containing protein 11) is an E3 ubiquitin ligase recently identified as an oncogene in malignant glioma and lung cancer. In the present study, we report that expression of TRIM11 was increased in colon cancer (CC) tissue relative to paired normal tissues and that higher TRIM11 levels predicted poor overall survival (OS) and disease-free survival (DFS) in CC patients. Mechanistically, we showed that miR-24-3p downregulation contributes to TRIM11 upregulation in CC. We also demonstrated that TRIM11 overexpression promotes cell proliferation and colony formation and inhibits apoptosis in CC, while knocking down TRIM11 using CRISPR/Cas9-mediated genome editing inhibited cell proliferation and induced apoptosis. Silencing TRIM11 in vivo decreased tumor growth. These findings indicate that TRIM11 facilitates CC progression by promoting cell proliferation and inhibiting apoptosis and that the novel miR-24-3p/TRIM11 axis may be a useful new target for treating patients with CC.

Yu, S., N. Parameswaran, et al. "CRABP-II enhances pancreatic cancer cell migration and invasion by stabilizing interleukin 8 expression." *Oncotarget*. 2016 Dec 26. doi: 10.18632/oncotarget.14194.

Our previous study shows that cellular retinoic acid binding protein II (CRABP-II) is overexpressed in pancreatic ductal adenocarcinoma (PDAC) and pre-cancerous lesions, but not detected in normal pancreatic tissues. In this study, we show that deletion of CRABP-II in PDAC cells by CRISPR/Cas9 does not affect cancer cell proliferation, but decreases cell migration and invasion. Gene expression microarray analysis reveals that IL-8 is one of the top genes whose expression is down-regulated upon CRABP-II deletion, while expression of MMP-2 and MMP-14, two targets of IL-8 are also significantly down-regulated. Moreover, we found that CRABP-II is able to form a complex with HuR, which binds to the 3'UTR of IL-8 messenger RNA (mRNA) and enhances IL-8 mRNA stability. Ectopic expression of

flag-CRABP-II in CRABP-II knockout cells is able to rescue the expression of IL-8, MMP-2/MMP-14 and recovers cell migration. Using the orthotopic xenograft model, we further demonstrate that CRABP-II deletion impairs tumor metastasis to nearby lymph nodes. Taken together, our results reveal a novel pathway linking CRABP-II expression to enhanced PDAC metastasis, and hence we propose CRABP-II may serve as a new PDAC therapeutic target.

Yuan, B., X. Wang, et al. "DHX33 Transcriptionally Controls Genes Involved in the Cell Cycle." Mol Cell Biol. 2016 Nov 14;36(23):2903-2917. Print 2016 Dec 1.

The RNA helicase DHX33 has been shown to be a critical regulator of cell proliferation and growth. However, the underlying mechanisms behind DHX33 function remain incompletely understood. We present original evidence in multiple cell lines that DHX33 transcriptionally controls the expression of genes involved in the cell cycle, notably cyclin, E2F1, cell division cycle (CDC), and minichromosome maintenance (MCM) genes. DHX33 physically associates with the promoters of these genes and controls the loading of active RNA polymerase II onto these promoters. DHX33 deficiency abrogates cell cycle progression and DNA replication and leads to cell apoptosis. In zebrafish, CRISPR-mediated knockout of DHX33 results in downregulation of cyclin A2, cyclin B2, cyclin D1, cyclin E2, cdc6, cdc20, E2F1, and MCM complexes in DHX33 knockout embryos. Additionally, we found the overexpression of DHX33 in a subset of non-small-cell lung cancers and in Ras-mutated human lung cancer cell lines. Forced reduction of DHX33 in these cancer cells abolished tumor formation in vivo. Our study demonstrates for the first time that DHX33 acts as a direct transcriptional regulator to promote cell cycle progression and plays an important role in driving cell proliferation during both embryo development and tumorigenesis.

Yuan, M., P. Wang, et al. "A Simple and Efficient Approach to Construct Mutant Vaccinia Virus Vectors." J Vis Exp. 2016 Oct 30;(116). doi: [10.3791/54171](https://doi.org/10.3791/54171).

The CRISPR-associated endonuclease Cas9 can edit particular genomic loci directed by a single guide RNA (gRNA). The CRISPR/Cas9 system has been successfully employed for editing genomes of various organisms. Here we describe a protocol for editing the vaccinia virus (VV) genome in the cytoplasm of VV-infected CV-1 cells using the RNA-guided Cas9. RNA-guided Cas9 induces double-stranded DNA breaks facilitating homologous recombination efficiently and specifically in the

targeted site of VV and a transgene can be incorporated into these sites by homologous recombination. By using a site-specific homologous vector with transgene(s), a NIL gene-deleted VV with the red fluorescence protein (RFP) gene incorporated in this region was generated with a successful recombination efficiency 10 times greater than that obtained from the conventional homologous recombination method. This protocol demonstrates successful use of RNA-guided Cas9 system to generate mutant VVs with enhanced efficiency.

Zhang, H., Z. Chen, et al. "TG2 and NF-kappaB Signaling Coordinates the Survival of Mantle Cell Lymphoma Cells via IL6-Mediated Autophagy." Cancer Res. 2016 Nov 1;76(21):6410-6423. Epub 2016 Aug 3.

Expression of the transglutaminase TG2 has been linked to constitutive activation of NF-kappaB and chemotherapy resistance in mantle cell lymphoma (MCL) cells. TG2 forms complexes with NF-kappaB components, but mechanistic insights that could be used to leverage therapeutic responses has been lacking. In the current study, we address this issue with the discovery of an unexpected role for TG2 in triggering autophagy in drug-resistant MCL cells through induction of IL6. CRISPR-mediated silencing of TG2 delayed apoptosis while overexpressing TG2 enhanced tumor progression. Under stress, TG2 and IL6 mediate enhanced autophagy formation to promote MCL cell survival. Interestingly, the autophagy product ATG5 involved in autophagosome elongation positively regulated TG2/NF-kappaB/IL6 signaling, suggesting a positive feedback loop. Our results uncover an interconnected network of TG2/NF-kappaB and IL6/STAT3 signaling with autophagy regulation in MCL cells, the disruption of which may offer a promising therapeutic strategy. Cancer Res; 76(21); 6410-23. (c)2016 AACR.

Zhang, Z., J. R. Christin, et al. "Mammary-Stem-Cell-Based Somatic Mouse Models Reveal Breast Cancer Drivers Causing Cell Fate Dysregulation." Cell Rep. 2016 Sep 20;16(12):3146-56. doi: [10.1016/j.celrep.2016.08.048](https://doi.org/10.1016/j.celrep.2016.08.048).

Cancer genomics has provided an unprecedented opportunity for understanding genetic causes of human cancer. However, distinguishing which mutations are functionally relevant to cancer pathogenesis remains a major challenge. We describe here a mammary stem cell (MaSC) organoid-based approach for rapid generation of somatic genetically engineered mouse models (GEMMs). By using RNAi and CRISPR-mediated genome engineering in MaSC-GEMMs, we have discovered that inactivation of Ptpn22 or Mll3, two genes mutated in human breast

cancer, greatly accelerated PI3K-driven mammary tumorigenesis. Using these tumor models, we have also identified genetic alterations promoting tumor metastasis and causing resistance to PI3K-targeted therapy. Both Ptpn22 and Mll3 inactivation resulted in disruption of mammary gland differentiation and an increase in stem cell activity. Mechanistically, Mll3 deletion enhanced stem cell activity through activation of the HIF pathway. Thus, our study has established a robust in vivo platform for functional cancer genomics and has discovered functional breast cancer mutations.

Zhen, S., L. Hua, et al. "Inhibition of long non-coding RNA UCA1 by CRISPR/Cas9 attenuated malignant phenotypes of bladder cancer." *Oncotarget*. 2016 Dec 25. doi: [10.18632/oncotarget.14176](https://doi.org/10.18632/oncotarget.14176).

CRISPR/Cas9 is a novel and effective genome editing technique, but its application is not widely expanded to manipulate long non-coding RNA (lncRNA) expression. The lncRNA urothelial carcinoma-associated 1 (UCA1) is upregulated in bladder cancer and promotes the progression of bladder cancer. Here, we design gRNAs specific to UCA1 and construct CRISPR/Cas9 systems targeting UCA1. Single CRISPR/Cas9-UCA1 can effectively inhibit UCA1 expression when transfected into 5637 and T24 bladder cancer cells, while the combined transfection of the two most effective CRISPR/Cas9-UCA1s can generate more satisfied inhibitory effect. CRISPR/Cas9-UCA1s attenuate UCA1 expression via targeted genome-specific DNA cleavage, resulting in the significant inhibition of cell proliferation, migration and invasion in vitro and in vivo. The mechanisms associated with the inhibitory effect of CRISPR/Cas9-UCA1 on malignant phenotypes of bladder cancer are attributed to the induction of cell cycle arrest at G1 phase, a substantial increase of apoptosis, and an enhanced activity of MMPs. Additionally, urinary UCA1 can be used as a non-invasive diagnostic marker for bladder cancer as revealed by a meta-analysis. Collectively, our data suggest that CRISPR/Cas9 technique can be used to down-modulate lncRNA expression, and urinary UCA1 may be used as a non-invasive marker for diagnosis of bladder cancer.

Zhen, S., J. J. Lu, et al. "In Vitro and In Vivo Synergistic Therapeutic Effect of Cisplatin with Human Papillomavirus16 E6/E7 CRISPR/Cas9 on Cervical Cancer Cell Line." *Transl Oncol*. 2016 Dec;9(6):498-504. doi: [10.1016/j.tranon.2016.10.002](https://doi.org/10.1016/j.tranon.2016.10.002). Epub 2016 Oct 28.

PURPOSE: Human papillomavirus (HPV) type 16 is one of the major etiologic factors of cervical cancer. Our study aims to investigate the potentiality of the antiviral clustered regularly interspaced short

palindromic repeat (CRISPR)/CRISPR-associated Cas9 system (CRISPR/Cas9) targeting the E6 and E7 oncogenes of HPV16 as a potential chemosensitizer of cisplatin (cis-diaminedichloroplatinum II; CDDP) for cervical cancer. METHODS: Specifically, the therapeutic efficacy of combination of CDDP and HPV16 E6 + E7-CRISPR/Cas9 was assessed in cervical cancer cells and cervical cancer xenograft models. RESULTS: In vitro experiments showed that long-term exposure of SiHa cells to the HPV16 E6 + E7-CRISPR/Cas9 induced apoptosis, and its pro-apoptosis effect became more obvious when combined with CDDP. In vivo study found the efficacy of the combination of HPV16 E6 + E7-CRISPR/Cas9 and CDDP were superior to either of the treatments in term of apoptosis induction and metastasis inhibition. CONCLUSION: Collectively, our results suggested that HPV16 E6 + E7-CRISPR/Cas9 could be an effective sensitizer of CDDP chemotherapy in cervical cancer.

Zhen, S., Y. Takahashi, et al. "Targeted delivery of CRISPR/Cas9 to prostate cancer by modified gRNA using a flexible aptamer-cationic liposome." *Oncotarget*. 2016 Dec 21. doi: [10.18632/oncotarget.14072](https://doi.org/10.18632/oncotarget.14072).

The potent ability of CRISPR/Cas9 system to inhibit the expression of targeted gene is being exploited as a new class of therapeutics for a variety of diseases. However, the efficient and safe delivery of CRISPR/Cas9 into specific cell populations is still the principal challenge in the clinical development of CRISPR/Cas9 therapeutics. In this study, a flexible aptamer-liposome-CRISPR/Cas9 chimera was designed to combine efficient delivery and increased flexibility. Our chimera incorporated an RNA aptamer that specifically binds prostate cancer cells expressing the prostate-specific membrane antigen as a ligand. Cationic liposomes were linked to aptamers by the post-insertion method and were used to deliver therapeutic CRISPR/Cas9 that target the survival gene, polo-like kinase 1, in tumor cells. We demonstrate that the aptamer-liposome-CRISPR/Cas9 chimeras had a significant cell-type binding specificity and a remarkable gene silencing effect in vitro. Furthermore, silencing promoted a conspicuous regression of prostate cancer in vivo. Importantly, the approach described here provides a universal means of cell type-specific CRISPR/Cas9 delivery, which is a critical goal for the widespread therapeutic applicability of CRISPR/Cas9 or other nucleic acid drugs.

Zhu, S., W. Li, et al. "Genome-scale deletion screening of human long non-coding RNAs using a paired-guide RNA CRISPR-Cas9 library." *Nat Biotechnol*. 2016 Dec;34(12):1279-1286. doi: [10.1038/nbt.3715](https://doi.org/10.1038/nbt.3715). Epub

2016 Oct 31.

CRISPR-Cas9 screens have been widely adopted to analyze coding-gene functions, but high-throughput screening of non-coding elements using this method is more challenging because indels caused by a single cut in non-coding regions are unlikely to produce a functional knockout. A high-throughput method to produce deletions of non-coding DNA is needed. We report a high-throughput genomic deletion strategy to screen for functional long non-coding RNAs (lncRNAs) that is based on a lentiviral paired-guide RNA (pgRNA) library. Applying our screening method, we identified 51 lncRNAs that can positively or negatively regulate human cancer cell growth. We validated 9 of 51 lncRNA hits using CRISPR-Cas9-mediated genomic deletion, functional rescue, CRISPR activation or inhibition and gene-expression profiling. Our high-throughput pgRNA genome deletion method will enable rapid identification of functional mammalian non-coding elements.

Zou, M., A. Bhatia, et al. "Evolutionarily conserved dual lysine motif determines the non-chaperone function of secreted Hsp90alpha in tumour progression." *Oncogene*. 2016 Oct 10. doi: [10.1038/onc.2016.375](https://doi.org/10.1038/onc.2016.375).

Both intracellular and extracellular heat shock protein-90 (Hsp90) family proteins (alpha and beta) have been shown to support tumour progression. The tumour-supporting activity of the intracellular Hsp90 is attributed to their N-terminal ATPase-driven chaperone function. What molecular entity determines the extracellular function of secreted Hsp90 and the distinction between Hsp90alpha and Hsp90beta was unclear. Here we demonstrate that CRISPR/Cas9 knocking out Hsp90alpha nullifies tumour cells' ability to migrate, invade and metastasize without affecting the cell survival and growth. Knocking out Hsp90beta leads to tumour cell death. Extracellular supplementation with recombinant Hsp90alpha, but not Hsp90beta, protein recovers tumourigenicity of the Hsp90alpha-knockout cells. Sequential mutagenesis identifies two evolutionarily conserved lysine residues, lys-270 and lys-277, in the Hsp90alpha subfamily that determine the extracellular Hsp90alpha function. Hsp90beta subfamily lacks the dual lysine motif and the extracellular function. Substitutions of gly-262 and thr-269 in Hsp90beta with lysines convert Hsp90beta to a Hsp90alpha-like protein. Newly constructed monoclonal antibody, 1G6-D7, against the dual lysine region of secreted Hsp90alpha inhibits both de novo tumour formation and expansion of already formed tumours in mice. This study suggests an alternative therapeutic approach to target Hsp90 in cancer, that is, the tumour-secreted Hsp90alpha, instead of the intracellular Hsp90alpha and Hsp90beta. *Oncogene*

advance online publication, 10 October 2016; doi:10.1038/onc.2016.375.

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