

Literature Introducing of Cancer and Animal Studies

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Abstract: Cancer is the cells that divide and grow uncontrollably, which form malignant tumors, and could exist in all parts of the human body. This material introduces some literature descriptions on the animal model application in the cancer researches.

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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.

Literatures

Ahn, W. S., S. M. Bae, et al. (2003). "A therapy modality using recombinant IL-12 adenovirus plus E7 protein in a human papillomavirus 16 E6/E7-associated cervical cancer animal model." *Hum Gene Ther* **14**(15): 1389-99.

Interleukin (IL)-12 has been reported to induce cellular immune responses for protection against tumor formation. In this paper published in 2003 in *Hum Gene Ther*, Ahn et al investigated the utility of adenoviral delivery of IL-12 as an adjuvant for a human papillomavirus E7 subunit vaccine in a mouse tumor challenge model. Their studies demonstrate that E7 vaccines can induce the cytotoxic T-lymphocyte (CTL) responses responsible for antitumor effects in the presence of IL-12 delivered via adenovirus vectors. This likely provides one additional approach for immune therapy against cervical cancers.

Blouin, S., M. F. Basle, et al. (2008). "Interactions between microenvironment and cancer cells in two animal models of bone metastasis." *Br J Cancer* **98**(4): 809-15.

The preferential proliferation of cancer cells in the bone microenvironment is poorly characterised. Interaction between stromal and cancer cells induces the expression on the formers of characteristics

leading to osteoclastogenesis only in the bone microenvironment.

Dwyer, R. M., S. M. Schatz, et al. (2005). "A preclinical large animal model of adenovirus-mediated expression of the sodium-iodide symporter for radioiodide imaging and therapy of locally recurrent prostate cancer." *Mol Ther* **12**(5): 835-41.

The sodium-iodide symporter (NIS) is primarily a thyroid protein, providing for the accumulation of iodide for biosynthesis of thyroid hormones. Native NIS expression has made possible the use of radioactive iodide to image and treat thyroid disease successfully. In 2005, Dwyer et al demonstrated the successful introduction of localized NIS expression in the prostate gland of dogs, with no vector-related toxicity observed. None of the animals experienced any surgical complications, and serum chemistry panels showed no significant change following therapy. The results presented provide further evidence of the safety and efficacy of NIS as a therapeutic gene and support translation of this work into the clinical setting.

Edelmann, L. and W. Edelmann (2004). "Loss of DNA mismatch repair function and cancer predisposition in the mouse: animal models for human hereditary nonpolyposis colorectal cancer." *Am J Med Genet C Semin Med Genet* **129C**(1): 91-9.

Germline mutations in DNA mismatch repair genes underlie one of the most common hereditary cancer predisposition syndromes known in humans, hereditary nonpolyposis colorectal cancer (HNPCC). Defects of the DNA mismatch repair system are also prevalent in sporadic colorectal cancers. The generation of mice with targeted inactivating mutations in the mismatch repair genes has facilitated the in vivo study of how these genes function and how their individual loss contributes to tumorigenesis. Although there are notable limitations when using murine models to study the molecular basis of human

cancer, there is remarkable similarity between the two species with respect to the contribution of individual members of the mismatch repair system to cancer susceptibility, and mouse mutants have greatly enhanced our understanding of the normal role of these genes in mutation avoidance and suppression of tumorigenesis.

Fasano, J. and F. Muggia (2009). "Breast cancer arising in a BRCA-mutated background: therapeutic implications from an animal model and drug development." *Ann Oncol* **20**(4): 609-14.

To date, the presence of a hereditary background has not influenced the selection of drug treatment in breast cancer. However, increasingly, negative hormone receptors and Her2 (often referred to as 'triple negative') or a medullary carcinoma histology has been reported in BRCA mutation carriers. Accordingly, such patients are often considered for adjuvant protocols based on chemotherapy (and not based on endocrine manipulations or trastuzumab). Mouse models introducing a conditional BRCA-null expression in the breast have recently provided powerful support for cisplatin-based treatment and have implications for the design of adjuvant studies in these patients.

Giuriato, S., K. Rabin, et al. (2004). "Conditional animal models: a strategy to define when oncogenes will be effective targets to treat cancer." *Semin Cancer Biol* **14**(1): 3-11.

The ability to model cancer in the mouse has provided a robust methodology to dissect the molecular etiology of cancer. These models serve as potentially powerful platforms to preclinically evaluate novel therapeutics. In particular, the recent development of strategies to conditionally induce the or knockout the function of genes in a tissue specific manner has enabled investigators to engineer mice to demonstrate that the targeted inactivation of specific oncogenes can be effective in inducing sustained regression of tumors. Thus, these animal models will be useful to define the specific genes that will be therapeutically useful to target for the treatment of particular human cancers.

Gleich, L. L., Y. Q. Li, et al. (2003). "Alloantigen gene therapy for head and neck cancer: evaluation of animal models." *Head Neck* **25**(4): 274-9.

Human trials of alloantigen gene therapy, using the class I major histocompatibility complex (MHC) HLA-B7, have demonstrated the potential efficacy of this treatment for head and neck cancer. Its mechanism remains unclear. An immune-competent mouse model of MHC gene therapy to test factors potentially important to the tumor response is needed.

Two cell lines were used, B4B8 cells that grow in Balb/c mice and SCC-VII cells that grow in C3H mice. The mouse MHC H2-K(b) was used as the therapeutic gene, because it is an alloantigen to both mice strains. Plasmids that encode the H2-K(b) cDNA were prepared, and the cell lines were transfected. Mice were injected subcutaneously with naive cells to determine the tumor kinetics and serve as controls. Mice were injected with H2-K(b) transfected cells and tumor growth was compared with controls. Mice that did not grow tumor were rechallenged with naive cells to assess for tumor immunity. Mice were injected with transfected and naive cells admixed to determine whether the concentration of the alloantigen is important. RESULTS: B4B8 tumors grew slowly, whereas SCC-VII tumors grew rapidly. Transfection with H2-K(b) plasmid prevented or inhibited tumor growth of both the B4B8 and SCC-VII tumors. This growth inhibition was independent of the number of cells injected. In the mice that did not grow tumor, tumor immunity was demonstrated after challenge with naive cells in both models. There was no relationship between induction of immunity and the timing of the challenge or initial cell quantity. The mice injected with a mixture of naive and transfected cells grew tumor, although growth was delayed in the B4B8 model. According to this study, the two mouse models can serve as a rapid and slow growing tumor model of alloantigen gene therapy. In addition, it was noted that initial tumor cell number is not a significant factor for predicting tumor response and demonstrated that in both of these models alloantigen gene therapy results in significant antitumor immunity.

Ishida, H., T. Nakata, et al. (2007). "A novel steroidal selective steroid sulfatase inhibitor KW-2581 inhibits sulfated-estrogen dependent growth of breast cancer cells in vitro and in animal models." *Breast Cancer Res Treat* **106**(2): 215-27.

IN 2007, Ishida et al screened a series of 17beta-(N-alkylcarbamoyl)-estra-1,3,5(10)trine-3-O-sulfamate derivatives, and describe here a potent and selective steroid sulfatase (STS) inhibitor with antitumor effects in breast cancer models in vitro and in vivo. In biochemical assays using crude enzymes isolated from recombinant Chinese hamster ovary cells expressing human arylsulfatases (ARSs), one of the best compounds, KW-2581, inhibited STS activity with an IC(50) of 4.0 nM, while > 1000-fold higher concentrations were required to inhibit the other ARSs. The failure to stimulate the growth of MCF-7 human breast cancer cells as well as in uteri in ovariectomized rats indicated the lack of estrogenicity of this compound. In MCF-7 cells transfected with the STS gene, termed MCS-2 cells, KW-2581 inhibited the growth of cells stimulated by estrone sulfate (E1S)

but also 5-androstene-3 β , 17 β -diol 3-sulfate (ADIOLS) and dehydroepiandrosterone 3-sulfate. We found that oral administration of KW-2581 inhibited both E1S- and ADIOLS-stimulated growth of MCS-2 cells in a mouse hollow fiber model. In a nitrosomethylurea-induced rat mammary tumor model, KW-2581 induced regression of E1S-stimulated tumor growth as effectively as tamoxifen or another STS inhibitor, 667 Coumate. Dose-response studies in the same rat model demonstrated that more than 90% inhibition of STS activity in tumors was necessary to induce tumor shrinkage. STS activity in tumors has well correlated with that in leukocytes, suggesting that STS activity in leukocytes could be used as an easily detectable pharmacodynamic marker. These findings demonstrate that KW-2581 is a candidate for development as a therapeutic agent for the treatment of hormone receptors-positive breast cancer.

Kaijzel, E. L., G. van der Pluijm, et al. (2007). "Whole-body optical imaging in animal models to assess cancer development and progression." *Clin Cancer Res* **13**(12): 3490-7.

Different optical-based imaging models were used to investigate tumor progression and metastasis with particular emphasis on metastasis to bone and bone marrow. In 2007, Kaijzel et al described how optical imaging could be used to follow important processes in tumor development and treatment response, including angiogenesis, apoptosis, and proteolysis. Finally, we discuss the translation of one optical imaging modality, near-IR fluorescence, from animal validation studies to applications in the clinic related to cancer management.

Kawakami, K., M. Kawakami, et al. (2004). "Nitric oxide accelerates interleukin-13 cytotoxin-mediated regression in head and neck cancer animal model." *Clin Cancer Res* **10**(15): 5264-70.

Receptors for interleukin-13 (IL-13R) are overexpressed on several types of solid cancers including glioblastoma, renal cell carcinoma, AIDS Kaposi's sarcoma, and head and neck cancer. Recombinant fusion proteins IL-13 cytotoxin (IL13-PE38QQR or IL13-PE38) have been developed to directly target IL-13R-expressing cancer cells. Although it has been found that IL-13 cytotoxin has a direct potent antitumor activity in vivo in nude mice models of human cancers, the involvement of indirect antitumor effector molecules such as nitric oxide (NO) is unknown. To address this issue, we assessed the effect of NO inhibitor N(omega)-monomethyl-L-arginine on IL-13 cytotoxin-mediated cytotoxicity and NO₂/NO₃ production in HN12 head and neck cancer cells. In addition, antitumor effects and NO levels in

HN12 and KCCT873 head and neck tumors xenografted s.c. in nude mice when treated with IL-13 cytotoxin were evaluated by tumor measurement, Western blot, and immunohistochemistry analyses. Pretreatment of animals with N(omega)-monomethyl-L-arginine significantly decreased the NO levels and IL-13 cytotoxin-mediated antitumor effects. In addition, depletion of macrophages, known to produce NO, also decreased antitumor activity of IL-13 cytotoxin. Based on these studies, we concluded that NO accelerates antitumor effect of IL-13 cytotoxin on head and neck tumor cells. Because IL-13 cytotoxin is currently being tested in the clinic for the treatment of patients with recurrent glioblastoma multiforme, our current findings suggest maintaining macrophage and NO-producing cellular function for optimal therapeutic effect of this targeted agent.

Kawakami, K., M. Kawakami, et al. (2004). "Specifically targeted killing of interleukin-13 (IL-13) receptor-expressing breast cancer by IL-13 fusion cytotoxin in animal model of human disease." *Mol Cancer Ther* **3**(2): 137-47.

Interleukin-13 receptor (IL-13R) alpha2 chain binds IL-13 with high affinity and can internalize after binding to ligand. We have exploited this property of IL-13Ralpha2 chain by receptor-targeted breast cancer therapy. Previous studies have demonstrated that in vivo intratumoral (i.t.) gene transfer of this chain followed by IL-13 cytotoxin [comprised of IL-13 and Pseudomonas exotoxin (IL13-PE38QQR)] therapy causes regression of established human tumors in xenografted models. Breast carcinoma cells do not express IL-13Ralpha2 chain and are resistant to the antitumor effect of IL-13 cytotoxin. To determine whether IL-13Ralpha2 chain can render sensitivity of breast cancer to IL-13 cytotoxin, we injected IL-13Ralpha2 plasmid in s.c. established tumors by i.t. route, followed by systemic or i.t. IL-13 cytotoxin administration. This combination approach showed profound antitumor activity against human breast tumors in xenografted immunodeficient mice. Interestingly, there was dominant infiltration of inflammatory cells in regressing tumors, which were identified to be macrophages producing nitric oxide (NO) and natural killer cells. The partial role of inducible nitric oxide synthase (iNOS)-positive macrophages was confirmed by in vivo macrophage depletion experiments. Serum chemistry, hematology, and organ histology from treated mice did not show any remarkable toxicity resulting from the combination therapy. Taken together, local gene transfer of IL-13Ralpha2 followed by receptor-targeted IL-13 cytotoxin therapy may be applied safely and effectively to the treatment of localized breast cancer.

Kim, S. Y., Y. S. Yang, et al. (2008). "Adenovirus-mediated expression of dominant negative c-myc induces apoptosis in head and neck cancer cells and inhibits tumor growth in animal model." *Oral Oncol* **44**(4): 383-92.

The demonstration of aberrant expression of the c-myc proto-oncogene in various cancers suggests that c-myc plays an important role in the development of cancer. On this basis, it has been proposed that ablation of c-myc function might be an effective approach for therapy of c-myc dependent malignancies. According to the study in 2008 by Kim et al, DN-myc was expressed in an adenovirus-mediated gene delivery system and introduced into head and neck squamous cell carcinoma cells (HNSCC) in vitro and in vivo to examine its tumor suppressive function and its potential in HNSCC gene therapy. Over expression of DN-myc in HNSCC cells inhibited in vitro cell proliferation, expression of growth factors such as IGF-I, -II, IGF-1R, and VEGF, inhibited Akt/PKB pathway activation, and enhanced induction of apoptosis. Similarly, in vivo administration of DN-myc retarded tumor-growth. Our results support a role for DN-myc in inducing apoptosis and tumor suppression, and, furthermore, suggest that DN-myc gene therapy might provide a powerful tool for treatment of c-myc dependent malignancies such as HNSCC.

Kwon, K. H., A. Barve, et al. (2007). "Cancer chemoprevention by phytochemicals: potential molecular targets, biomarkers and animal models." *Acta Pharmacol Sin* **28**(9): 1409-21.

The cancer-protective effects elicited by these dietary compounds are believed to be due at least in part to the induction of cellular defense systems including the detoxifying and antioxidant enzymes system, as well as the inhibition of anti-inflammatory and anti-cell growth signaling pathways culminating in cell cycle arrest and/or cell death. In this review, we summarize the potential mechanisms including the modulation of nuclear factor kappaB (NF-kappaB), cyclooxygenases-2 (COX-2), activator protein-1 (AP-1), mitogen-activated protein kinases (MAPKs) and the induction of phase II cellular detoxifying and antioxidant enzymes mediated mainly by the antioxidant response elements (ARE) within the promoter regions of these genes through nuclear factor-erythroid 2-related factor 2 (Nrf2), a member of the Cap "n" collar (CNC) family of the basic region-leucine zipper transcription factor. In addition, we also review several animal models of carcinogenesis and cancer chemopreventive efficacy studies of these animal models using dietary chemopreventive compounds. Finally, we discuss the cellular signaling

casades mediated by Nrf2, NF-kappaB, AP-1, MAPKs and COX-2, which have been considered to play pivotal roles in tumor initiation, promotion and progression processes, and could be promising molecular targets for the design of drugs targeting cancer prevention and therapy.

Lai, M. D., M. C. Yen, et al. (2009). "The effects of DNA formulation and administration route on cancer therapeutic efficacy with xenogenic EGFR DNA vaccine in a lung cancer animal model." *Genet Vaccines Ther* **7**: 2.

Tyrosine kinase inhibitor gefitinib is effective against lung cancer cells carrying mutant epidermal growth factor receptor (EGFR); however, it is not effective against lung cancer carrying normal EGFR. The breaking of immune tolerance against self epidermal growth factor receptor with active immunization may be a useful approach for the treatment of EGFR-positive lung tumors. Xenogenic EGFR gene was demonstrated to induce antigen-specific immune response against EGFR-expressing tumor with intramuscular administration. In order to enhance the therapeutic effect of xenogenic EGFR DNA vaccine, the efficacy of altering routes of administration and formulation of plasmid DNA was evaluated on the mouse lung tumor (LL2) naturally overexpressing endogenous EGFR in C57B6 mice. Three different combination forms were studied, including (1) intramuscular administration of non-coating DNA vaccine, (2) gene gun administration of DNA vaccine coated on gold particles, and (3) gene gun administration of non-coating DNA vaccine. LL2-tumor bearing C57B6 mice were immunized four times at weekly intervals with EGFR DNA vaccine. RESULTS: The results indicated that gene gun administration of non-coating xenogenic EGFR DNA vaccine generated the strongest cytotoxicity T lymphocyte activity and best antitumor effects. CD8(+) T cells were essential for anti-tumor immunity as indicated by depletion of lymphocytes in vivo. Thus, our data demonstrate that administration of non-coating xenogenic EGFR DNA vaccine by gene gun may be the preferred method for treating EGFR-positive lung tumor in the future.

Lamb, D. J. and L. Zhang (2005). "Challenges in prostate cancer research: animal models for nutritional studies of chemoprevention and disease progression." *J Nutr* **135**(12 Suppl): 3009S-3015S.

Prostate cancer is the second leading cause of cancer-related death in the United States. The American Cancer Society estimates that there will be over 232,000 new cases of prostate cancer in 2005. Evidence suggests that diet can act as a chemopreventive agent to reduce the incidence of

prostate cancer as well as to reduce the mortality of the disease. Epidemiologic studies suggest that diets rich in specific vitamins, grains, fruits, and vegetables may be associated with lower cancer rates than high-fat diets, yet the molecular bases for these positive nutritional actions are largely unknown. The interactions of diet in combination with genetic determinants of disease progression are unclear because prostate cancer is also a disease resulting from abnormal gene expression. Hence, the biology of normal prostate development and the mechanisms underlying the initiation, progression, and metastatic spread of prostate cancer must be understood at the molecular level to develop effective nutritional prevention and intervention strategies to control and treat this malignant disease. However, progress toward understanding the biology of prostate cancer and the development of new therapies has been hampered by the lack of in vivo model systems that adequately recapitulate the spectrum of benign, latent, aggressive, and metastatic forms of the human disease. In this review we discuss the diverse animal models of prostate cancer available and their applicability for nutritional studies of cancer prevention.

Liao, Y., Y. Y. Zou, et al. (2004). "Enhanced paclitaxel cytotoxicity and prolonged animal survival rate by a nonviral-mediated systemic delivery of E1A gene in orthotopic xenograft human breast cancer." *Cancer Gene Ther* **11**(9): 594-602.

Paclitaxel (Taxol) is a promising frontline chemotherapeutic agent for the treatment of human breast and ovarian cancers. The adenoviral type 5 E1A gene has been tested in multiple clinical trials for its anticancer activity. E1A has also been shown to sensitize paclitaxel-induced killing in E1A-expressing cells. Here, we show that E1A can sensitize paclitaxel-induced apoptosis in breast cancer cells in a gene therapy setting by an orthotopic mammary tumor model. According to the study in 2004, Liao et al showed that expression of E1A enhanced in vitro paclitaxel cytotoxicity, as compared to the control cells. They compared the therapeutic efficacy of paclitaxel between orthotopic tumor models established with vector-transfected MDA-MB-231 (231-Vect) versus 231-E1A stable cells, using tumor weight and apoptotic index (TUNEL assay) as the parameters. And, Liao et al found paclitaxel was more effective in shrinking tumors and inducing apoptosis in tumor models established with stable 231-E1A cells than the control 231-Vect cells. The E1A gene therapy indeed enhances the sensitivity of tumor cells to chemotherapy in a gene therapy setting and, the current study provides preclinical data to support combination therapy between E1A gene and chemotherapy for future clinical trials.

McLarty, K., A. Fasih, et al. (2009). "18F-FDG small-animal PET/CT differentiates trastuzumab-responsive from unresponsive human breast cancer xenografts in athymic mice." *J Nucl Med* **50**(11): 1848-56.

Breast cancers (BCs) with high human epidermal growth factor receptor type 2 (HER2) expression are most likely to respond to trastuzumab; however, the mechanisms of action of trastuzumab are complex and there are no established biomarkers to accurately monitor treatment outcome in individual patients. Therefore, our aim was to determine, in human BC xenografts in athymic mice treated with trastuzumab, whether there were any changes in (18)F-FDG uptake that were associated with response to the drug and that could have utility in monitoring response in patients. Baseline tumor uptake of (18)F-FDG was measured in mice with MDA-MB-361 HER2-overexpressing xenografts and MDA-MB-231 xenografts with low HER2 expression by small-animal PET imaging on day 0. Mice were treated with phosphate-buffered saline (PBS) or trastuzumab (4 mg/kg), and small-animal PET was repeated 2 d after treatment. Maintenance doses of trastuzumab (2 mg/kg) or PBS were administered on days 7 and 14, and mice were imaged again on days 9 and 16. Tumor uptake was measured as percentage injected dose per gram (%ID/g) by volume-of-interest analysis on days 0 (baseline), 2, 9, and 16, followed by biodistribution studies on day 16. Tumor growth was measured, and a tumor growth index was calculated. RESULTS: The treatment of mice with trastuzumab, compared with control mice treated with PBS, resulted in a significant decrease in tumor uptake of (18)F-FDG in HER2-overexpressing MDA-MB-361 xenografts after 16 d of treatment (2.6 +/- 0.8 %ID/g vs. 4.6 +/- 1.8 %ID/g, respectively; $P < 0.03$) but not after 2 or 9 d of treatment ($P = 0.28-0.32$). In contrast, there was no significant change in the tumor uptake of MDA-MB-231 xenografts with low HER2 expression during the entire course of therapy (4.4 +/- 1.7 %ID/g vs. 3.6 +/- 1.1 %ID/g, respectively; $P = 0.31$). Trastuzumab treatment, compared with PBS treatment of controls, resulted in significant growth inhibition of MDA-MB-361 xenografts as early as 10 d from the initiation of treatment (tumor growth index, 0.7 +/- 0.2 vs. 1.7 +/- 0.3, respectively; $P < 0.0005$), whereas no tumor growth inhibition was observed for MDA-MB-231 xenografts (5.3 +/- 2.7 and 5.2 +/- 3.0; $P = 0.95$). As the conclusion by McLarty et al, changes in the tumor uptake of (18)F-FDG after therapy accurately identified responding and nonresponding human BC xenografts in athymic mice treated with trastuzumab; however, diminished glucose utilization did not precede changes in tumor volume.

Nemeth, A., E. Nadasi, et al. (2003). "Early effects of different cytostatic protocols for head and neck cancer on oncogene activation in animal experiments." *Anticancer Res* **23**(6C): 4831-5.

In vivo investigations on oncogenes and onco-suppressor genes may provide new findings on the potential carcinogenic effects of various cytostatic protocols inducing secondary tumours of the head and neck. Further surgeries are often necessary due to regional recurrence after the Cisplatin-supplemented BVM (Bleomycin, Vincristine, Methotrexate) protocol in the treatment of human head and neck tumours. Our earlier studies have illustrated the carcinogenic and mutagenic potential of Cisplatin. The effect of Cisplatin on the alteration of different onco- and suppressor genes has also been proven. Our present study aimed at investigating the early effects of the BVM and the CFu (Cisplatin, 5-Fluorouracil) protocols on early oncogene and tumour suppressor gene expressions in mice. Body weight equivalent amounts of cytostatics were administered intraperitoneally to 6- to 8-week-old, inbred, female CBA/Ca mice. Twenty-four, 48 and 72 hours after the treatment, RNA was isolated from the target organs and the quantitative expression of c-myc, Ha-ras and p53 genes were examined. The protocols caused detectable changes. A "short-term" in vivo test, the 24-hour examination of gene expression, is suitable for detecting early effects of carcinogen exposure. The alterations of gene expression, caused by the Cisplatin-containing protocol, draw attention to the probable role of Cisplatin in the development of regional recurrence and to the possibility of prevention.

Ostrand-Rosenberg, S. (2004). "Animal models of tumor immunity, immunotherapy and cancer vaccines." *Curr Opin Immunol* **16**(2): 143-50.

Reliable animal models are critical for evaluating immunotherapies and for defining tumor immunology paradigms. Tumor immunologists are moving away from traditional transplantation tumor systems because they do not adequately model human malignancies. Transgenic mouse models in which tumors arise spontaneously have been developed for most cancers. The models use one of three technologies: tissue-specific promoters to drive expression of SV40 large T antigen or tissue-specific oncogenes; deletion of tumor suppressor genes by gene targeting; or, conditional deletion of tumor suppressor genes or activation of oncogenes via Cre-lox technology. Knockin mice expressing human tumor antigens and gene-targeted mice with deletions for immunologically relevant molecules have been integral to advancing knowledge of the tumor-host relationship. Although animal models are becoming

more sophisticated, additional improvements are needed so that more realistic models can be developed.

Pantazis, P., Z. Han, et al. (2003). "Camptothecin and 9-nitrocamptothecin (9NC) as anti-cancer, anti-HIV and cell-differentiation agents. Development of resistance, enhancement of 9NC-induced activities and combination treatments in cell and animal models." *Anticancer Res* **23**(5A): 3623-38.

The anticancer drug, 9-nitrocamptothecin (9NC), has demonstrated an unprecedented activity against human cancer cells grown in cultures and as xenografts in nude mice. 9NC-induced apoptosis of cancer cells is mediated by the nuclear enzyme, topoisomerase I, and executed by pathways that involve cytochrome c release from the mitochondrion and/or activation of death receptors depending on the cell type. Alternatively, 9NC has exhibited ability to induce differentiation or senescence of certain cell types in vitro. In several instances, the 9NC activities can be regulated by Bcl-2 family proteins and cell cycle-associated proteins, p53, p21 and Cdks. Also, 9NC can inhibit HIV replication in infected T- and monocytic cells in vitro. Development of resistance to 9NC, associated with mutations in the topoisomerase I gene, can be overcome by regulating specific proteins, such as RKIP, other than topoisomerase I. Finally, derivatives (i.e., alkyl esters) of 9NC, liposome-encapsulated 9NC and combined treatment of 9NC with ionizing radiation or hyperthermia are other approaches to enhance the apoptotic activity of 9NC against human cancer cells.

Park, H. C., J. Seong, et al. (2005). "Alteration of cancer pain-related signals by radiation: proteomic analysis in an animal model with cancer bone invasion." *Int J Radiat Oncol Biol Phys* **61**(5): 1523-34.

Although radiotherapy is highly effective in relieving bone pain due to cancer invasion, its mechanism remains unclear. A hind paw model of cancer pain was developed by transplanting a murine hepatocarcinoma, HCa-1, into the periosteal membrane of the foot dorsum of C3H/HeJ mice. Bone invasion from HCa-1 was histopathologically confirmed from sequential tumor sampling. For three experimental groups, a control (N), tumor without radiation (T), and tumor with radiation (TR), the development and level of pain were objectively examined in mice with a growing tumor by assessing pain-associated behavior. The differential expression of pain-related signals in the spinal cord was analyzed by proteomic analysis using high-resolution two-dimensional gel electrophoresis (2-DE) and mass spectrometry, and those of proteins by Western

blotting. The pain-mediating neurotransmitters in the spinal cord were also examined by immunohistochemical staining for calcitonin gene-related peptide (CGRP) and substance P. In the histopathologic examinations, bone invasion from HCa-1 was seen from Day 7 and was evident at Day 14 after transplantation, and measurable pain-associated behaviors were developed from Day 7. After 25 Gy of radiation to the tumors, the objective level of pain in the TR group decreased, with higher thresholds to mechanical and thermal stimulation than in the T group. From the 2-DE of spinal cord, 107 spots were identified; 12 proteins were changed more than fivefold because of tumor formation but then reversed after radiation in the tumor-bearing mice. The proteins involved included secretagogin, syntenin, P2X purinoreceptor 6 (P2X6), and Ca(2+)/Calmodulin-dependent protein kinase 1 (CaM kinase 1), the functions of which have been known to be involved in the Ca(2+)-signaling cascade, ATP-mediated fast synaptic transmission, or control of vesicular trafficking. Validations using Western blotting were successful for the CaM kinase and P2X6. In immunohistochemical staining of the spinal cord, a significant decrease after irradiation was shown in the expression of CGRP, but not in substance P. In 2005, Park et al developed a novel model for bone pain due to cancer invasion, which was confirmed by histopathologic examination and measurable pain-associated behaviors. Radiotherapy decreased the objective level of pain. The underlying mechanism seems to be related to the Ca(2+)-signaling cascade or control of vesicular trafficking.

Reis, L. O., T. C. Pereira, et al. (2009). "Experimental animal model and RNA interference: a promising association for bladder cancer research." World J Urol **27**(3): 353-61.

Animal models are at the centre of laboratory bladder cancer (BC) research and at the same time, the bridge to the clinic. A new and very promising therapeutic approach is to silence abnormally up-regulated genes in cancer, through small interfering RNA (siRNA) molecules. Therapeutic use and success of siRNAs will largely depend on their efficient and safe in vivo delivery and on avoiding accidental off-target effects. Intravesical siRNA is a strategy which may be the best deliver option to superficial BC like intravesical immunotherapy. Its direct action might allow a continuous intracellular exposure to effective siRNA concentrations. While the procedure of transurethral siRNA administration is promising for BC research allowing detection of new targets in BC therapy, the optimal intravesical carrier and the best target(s) to siRNA are to be determined.

Seong, J., H. C. Park, et al. (2004). "Radiation-induced alteration of pain-related signals in an animal model with bone invasion from cancer." Ann N Y Acad Sci **1030**: 179-86.

Although radiotherapy is highly effective in relieving bone pain from cancer invasion, the mechanism of pain relief remains unclear. To explore the mechanism of radiotherapy-induced analgesia, we have developed an animal model of bone pain resulting from cancer invasion. Using this animal model system, radiation-induced pain response and pain-related signals in the spinal cord were analyzed. The hind paw model of bone pain from cancer invasion was developed by injecting transplantable hepatocellular carcinoma, HCa-1, into the periosteal membrane of the foot dorsum in C3H/HeJ mice. Bony invasion from HCa-1 cells was confirmed by histopathological examinations. In 2004, Seong et al measured the development of pain-associated behaviors. In this model, changes in the objective level of pain response after irradiation of the tumor were analyzed. Expression of pain-related host signals in the spinal cord, such as calcitonin gene-related peptide (CGRP), substance P, and c-fos, was investigated with immunohistochemical staining. In the histopathological examinations, bone invasion from HCa-1 cells was seen from day 7 and was evident at day 14 after injection. Measurable pain-associated behaviors were developed from day 7. In this model, mice treated with radiotherapy showed decreased objective levels of pain with a higher threshold to graded mechanical stimulation than did control mice from day 3 after irradiation. After irradiation of tumors, significant decreases in the expression of CGRP were shown in the spinal cord, whereas neither substance P nor c-fos showed any alteration. We developed a novel hind paw model of bone pain from cancer invasion that was confirmed by histopathological examination and measurable pain-associated behaviors. Radiotherapy decreased the objective level of pain and the underlying mechanism involved in the alteration of pain-related host signal, CGRP, in the spinal cord.

Shan, W. and J. Liu (2009). "Epithelial ovarian cancer: focus on genetics and animal models." Cell Cycle **8**(5): 731-5.

Despite rapid advances in understanding ovarian cancer etiology, epithelial ovarian cancer remains the most lethal form of gynecologic cancers in the United States. The four morphologically-defined epithelial ovarian cancer subtypes--serous, endometrioid, mucinous, and clear cell carcinomas--are generally believed to originate from ovarian epithelial cells. Although it remains unclear how this single cell layer gives rise to morphologically distinct

cancers, it has been suggested that early genetic events may direct the differentiation of ovarian epithelial cells. A number of genetic alterations are frequently encountered during ovarian tumorigenesis, including oncogenic activities of KRAS, BRAF and AKT, and silencing mutations of TP53, RB and PTEN. However, knowledge about how these genetic elements are coordinated during ovarian cancer initiation and progression is very limited. The establishment of cell-culture systems and rodent-based models has made big strides towards a better understanding of the genetic bases of human epithelial ovarian tumorigenesis. More importantly, the rise of genetically-engineered rodent and human models, particularly in the past five years, has provided key insight in the role of specific genes during ovarian tumorigenesis. In this review, we offer a comprehensive coverage of currently-available in vitro and in vivo models of human epithelial ovarian cancer, focusing on latest updates of genetically-modified rodent and human models and the valuable information conveyed by them.

Sierra, A. (2009). "Animal models of breast cancer for the study of pathogenesis and therapeutic insights." Clin Transl Oncol **11**(11): 721-7.

Activation of oncogenes and inactivation of tumour suppressor genes are common events during breast cancer initiation and progression and often determine treatment responsiveness. Indeed, these events need to be recreated in in vitro systems and in mouse cancer models in order to unravel the molecular mechanisms involved in breast cancer initiation and metastasis and assess their possible impact on responses to anticancer drugs. Optical-based imaging models are used to investigate and to follow important tumour progression processes. Moreover, the development of novel anticancer strategies requires more sensitive and less invasive methods to detect and monitor in vivo drug responses in breast cancer models. This review highlights some of the current strategies for modelling breast cancer in vitro and in the mouse, in order to answer biological or translational questions about human breast malignancies.

Stell, A., A. Biserni, et al. (2007). "Cancer modeling: modern imaging applications in the generation of novel animal model systems to study cancer progression and therapy." Int J Biochem Cell Biol **39**(7-8): 1288-96.

Cancer is the result of a series of genetic and epigenetic mutations that evolve over years even decades and lead to the transformed phenotype. Paradoxically, most methods developed to study these changes are static and do not provide insights on the

dynamics of the sequela of steps involved in tumorigenesis. This major shortcoming now can be overcome with the application of reporter genes and imaging technologies, which are providing tools to examine specific molecular events and their role in the carcinogenic process in single cells. In the last decade reporter-based biosensors were created to study gene transcription, protein/protein interactions, sub-cellular trafficking and protease activities; this wealth of systems enable to monitor intracellular signaling pathways at several key check points specifically involved in cancer cell development. The challenge is now to extend cell-based models to the generation of reporter mice, where non-invasive in vivo imaging technologies allow to follow single molecular events. When combined with murine models of cancer, these technologies will give an unprecedented opportunity to spatio-temporally investigate the molecular events resulting in neoplasia. The aim of the present review is to highlight the major changes occurring in this rapidly evolving field and their potential for increasing our knowledge in cancer biology and for the research of novel and more efficacious therapies.

Telang, N. and M. Katdare (2007). "Cell culture model for colon cancer prevention and therapy: an alternative approach to animal experimentation." Altex **24**(1): 16-21.

Mouse models for colon cancer that harbor a germ line mutation in the tumor suppressor gene Adenomatous polyposis coli (Apc) exhibit a primary genetic defect that predisposes to a high incidence of adenomatous polyps in the small intestine rather than in the colon. Colon cell culture models expressing quantifiable markers for carcinogenic risk may represent an alternative approach to reduce, refine or replace long-term animal experimentation. The newly developed colon epithelial cell lines 1638N COL-CI(1) (clonal derivative of the parental Apc mutant cell line 1638N COL) and 1638N COL-Pr(1) (tumor derivative of the clone), established from an Apc1638N [+/-] mutant mouse, exhibit aberrant cell cycle progression, downregulated apoptosis, enhanced carcinogenic risk and tumor formation, indicating that aberrantly proliferative preneoplastic 1638N COL-CI(1) cells exhibit a quantifiable risk for carcinogenesis. Treatment of these preneoplastic Apc mutant cells with a combination of celecoxib and 5-fluorouracil at clinically achievable low concentrations produced a 2.1 fold to 5.5 fold higher efficacy for cytostatic growth arrest and a 40.2% to 52.4% higher efficacy for inhibition of carcinogenic risk, relative to that obtained by these agents used individually. Thus, a low dose combination of mechanistically distinct agents resulted in enhanced efficacy. These data validate a novel cell culture

model and a rapid mechanism-based approach to prioritize efficacious drug combinations for animal studies and clinical trials on cancer prevention and, thereby, support the 3R concept by refining and/or reducing the use of animals in biomedical research relevant to prevention/therapy of colon cancer.

Tseng, J. C., A. Hurtado, et al. (2004). "Using sindbis viral vectors for specific detection and suppression of advanced ovarian cancer in animal models." *Cancer Res* **64**(18): 6684-92.

In 2004, Tseng et al studied the therapeutic value of Sindbis vectors for advanced metastatic ovarian cancer by using two highly reproducible and clinically accurate mouse models: a SCID xenograft model, established by i.p. inoculation of human ES-2 ovarian cancer cells, and a syngenic C57BL/6 model, established by i.p. inoculation of mouse MOSEC ovarian cancer cells. We demonstrate through imaging, histologic, and molecular data that Sindbis vectors systemically and specifically infect/detect and kill metastasized tumors in the peritoneal cavity, leading to significant suppression of the carcinomatosis in both animal models. Use of two different bioluminescent genetic markers for the IVIS Imaging System permitted demonstration, for the first time, of an excellent correlation between vector delivery and metastatic locations in vivo. Sindbis vector infection and growth suppression of murine MOSEC tumor cells indicate that Sindbis tumor specificity is not attributable to a species difference between human tumor and mouse normal cells. Sindbis virus is known to infect mammalian cells using the Mr 67,000 laminin receptor. Immunohistochemical staining of tumor cells indicates that laminin receptor is elevated in tumor versus normal cells. Down-regulated expression of laminin receptor with small interfering RNA significantly reduces the infectivity of Sindbis vectors. Tumor overexpression of the laminin receptor may explain the specificity and efficacy that Sindbis vectors demonstrate for tumor cells in vivo. We show that incorporation of antitumor cytokine genes such as interleukin-12 and interleukin-15 genes enhances the efficacy of the vector. These results suggest that Sindbis viral vectors may be promising agents for both specific detection and growth suppression of metastatic ovarian cancer.

Tsuda, H., K. Fukamachi, et al. (2005). "High susceptibility of human c-Ha-ras proto-oncogene transgenic rats to carcinogenesis: a cancer-prone animal model." *Cancer Sci* **96**(6): 309-16.

Transgenic animals carrying human c-Ha-ras proto-oncogene, v-Ha-ras transgenic mice, pim-1 transgenic mice and several knockout mice deficient

of tumor suppressor genes, such as p53, have been shown to exhibit increased carcinogen susceptibility. As a result, studies into practical application and medium-term screening of environmental carcinogens are under way. Given the advantages of rat models characterized by larger organ size, abundant information regarding preneoplasias and virus-free constitution, we have concentrated on the generation of transgenic rats bearing copies of the human c-Ha-ras proto-oncogene and shown the Hras128 strain to be extremely sensitive to the induction of mammary carcinomas, and to a lesser extent, lesions in the urinary bladder, esophagus and skin. In most, if not all, the mammary cancers mutations of the transgene but not the endogenous H-ras gene are present, appearing to occur early in the process of tumorigenesis, which involves proliferation of cells in TEB and intraductal hyperplasia before carcinomas arise. Preliminary findings suggest that this is independent of endogenous ovarian hormones, although inhibited by soy isoflavones and promoted by atrazine and nonylphenols. Although further studies of the mechanisms are clearly necessary, the model appears to have great potential for screening purposes, not only for modifiers active in the breast, but also other organs where tumors characterized by ras gene mutations develop.

Welsh, J. (2004). "Vitamin D and breast cancer: insights from animal models." *Am J Clin Nutr* **80**(6 Suppl): 1721S-4S.

1alpha,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], the biologically active form of vitamin D that interacts with the vitamin D receptor (VDR), is a coordinate regulator of proliferation, differentiation, and survival of breast cancer cells. Therefore, vitamin D compounds that bind and activate VDRs offer promise as therapeutic agents for the treatment of established breast cancer. In addition, epidemiologic, clinical, and animal studies suggested that vitamin D status is important for protection against the development of breast cancer. To elucidate potential biological mechanisms through which vitamin D status might be associated with breast cancer risk, basic research studies focused on defining the molecular effects of vitamin D signaling in the normal mammary gland. Both VDR and vitamin D 1-hydroxylase, the enzyme that generates 1,25(OH)₂D₃, are expressed and dynamically regulated in the normal mammary gland. Furthermore, studies with mice lacking VDRs established that vitamin D participates in negative growth control of the normal mammary gland and that disruption of VDR signaling is associated with abnormal ductal morphologic features, increased incidence of preneoplastic lesions, and accelerated mammary tumor development. These

studies support the concept that suboptimal generation of 1,25(OH)₂D₃ in the mammary gland might sufficiently deregulate VDR-mediated gene expression to sensitize mammary cells to transformation. In light of these observations, studies to define the most appropriate biomarkers of vitamin D status in relation to protection against breast cancer among human subjects are urgently needed.

Funabashi, H., K. Uchida, et al. (2001). "Establishment of a Tcrb and Trp53 genes deficient mouse strain as an animal model for spontaneous colorectal cancer." *Exp Anim* **50**(1): 41-7.

A congenic C57BL/6JJcl-Tcrbtm1MomTrp53tm1 (Tcrb^{-/-}:Trp53^{-/-}) mouse lacking T-cell receptor beta chain (TCR beta) and transformation related protein 53 (p53) has been established at the N8th generation of backcrossing male Tcrb^{-/-}:Trp53^{-/-} mice, which had been obtained by mating a Tcrb^{-/-} mouse with a Trp53^{-/-} mouse, with female C57BL/6JJcl mice. In the mice deficient for the both genes, occurrence of tumor masses was observed mostly in the cecum with high frequency as examined at 3 months of age. The majority of the masses had histologic features of hyperplasia or dysplasia while occasional lesions were noted to be adenocarcinomas invading the submucosa (invasive adenocarcinoma). As examined at 4 months of age and thereafter, all mice had 4-5 colorectal tumors per animal, the lesions being located mainly in the cecum and, histopathologically, all the obvious neoplastic growths in the regions examined were invasive adenocarcinomas. The Tcrb and Trp53 genes deficient mouse strain which develops spontaneous colorectal carcinoma with fairly high frequency at early age would be useful as an animal model for colorectal cancer.

Gao, X. and Z. Nawaz (2002). "Progesterone receptors - animal models and cell signaling in breast cancer: Role of steroid receptor coactivators and corepressors of progesterone receptors in breast cancer." *Breast Cancer Res* **4**(5): 182-6.

Progesterone, an ovarian steroid hormone, plays a key role in the development and function of the mammary gland, as it also does in the uterus and the ovary. The action of progesterone is mediated through its intracellular cognate receptor, the progesterone receptor (PR), which functions as a transcription factor that regulates gene expression. As with other nuclear receptors, coregulators (coactivators and corepressors) recruited by the liganded or unliganded PR, either to enhance or to suppress transcription activity, modulate the function of the PR. Mutation or aberrant expression of the coregulators might thus affect the normal function of

the PR and hence disrupt the normal development of the mammary gland, which may lead to breast cancer.

Hsieh, C. L. and L. W. Chung (2001). "New prospectives of prostate cancer gene therapy: molecular targets and animal models." *Crit Rev Eukaryot Gene Expr* **11**(1-3): 77-120.

Prostate cancer is the most common cancer and the second leading cause of cancer-related death among North American men. The low cure rate for prostate cancer is associated with the fact that many patients have metastatic disease at the time of disease presentation. Currently available therapeutic modalities for prostate cancer, such as surgery, radiation, hormone therapy, and chemotherapy, have failed to cure patients because these therapies are not sufficiently tumor-specific, resulting in dose-limiting toxicity. Therefore, gene therapy may offer great promise in this regard. In this article, we summarize current advances in gene therapy technologies for the treatment of cancer in general, and future prospects for treatment of human prostate cancer metastasis. We specifically emphasize current studies for improvement, both in the efficiency and the specificity of viral and nonviral vectors, and restricted transgene expression in tumors, to achieve selective targeting with minimized host organ toxicity, based on the molecular understanding of potential regulatory differences between normal and tumor cells. Cell and animal models used to study prostate cancer growth, invasion, and metastasis, and their usefulness in preclinical evaluation of therapeutic vectors in the treatment of prostate cancer skeletal metastasis are also discussed.

Jarnagin, W. R., K. Delman, et al. (2000). "Neoadjuvant interleukin-12 immunogene therapy protects against cancer recurrence after liver resection in an animal model." *Ann Surg* **231**(5): 762-71.

In 2000, Jarnagin et al did the study to evaluate the neoadjuvant use of a herpes simplex viral (HSV) amplicon vector expressing the murine interleukin-12 (IL-12) gene. In Jarnagin et al study, surgery is the most effective therapy for hepatic malignancy. Recurrences, which are common, most often occur in the remnant liver and are due partly to growth of residual microscopic disease in the setting of postoperative host cellular immune dysfunction. The authors hypothesized that engineering tumors to secrete IL-12 in vivo would elicit an immune response directed at residual tumor and would reduce the incidence of recurrence after resection. Solitary hepatomas were established in Buffalo rat livers and directly injected with 10⁶ particles of HSV carrying the gene for IL-12, lacZ (beta-galactosidase) or with saline. One week after injection, the animals were

challenged with an intraportal injection of 106 tumor cells, with subsequent resection of the hepatic lobe containing the previously established macroscopic tumor nodule, recreating the clinical scenario of residual microscopic cancer. Hepatoma cells transfected with HSV-IL-12 produced high levels of IL-12 in vitro and in vivo. A significant local immune response developed, as evidenced by a progressive increase in the number of CD4(+) and CD8(+) lymphocytes in the tumor. Treatment of established hepatomas with HSV-IL-12 protected against growth of microscopic residual cancer after hepatic resection. Sixty-four percent of the animals treated with HSV-IL-12 had zero or one tumors compared with 30% of HSVlac-treated and 24% of saline-treated animals. This neoadjuvant immune strategy may prove useful in reducing the incidence of cancer recurrence after hepatic resection.

Kawakami, K., M. Kawakami, et al. (2001). "Interleukin-13 receptor-targeted cancer therapy in an immunodeficient animal model of human head and neck cancer." *Cancer Res* **61**(16): 6194-200.

Although interleukin-13 receptors (IL-13R) are overexpressed on several head and neck cancer cell lines, a majority of cell lines express only low levels of IL-13R. We have found that the primary interleukin-13-binding protein IL-13Ralpha2 chain plays an important role in ligand binding and internalization. We showed that the gene transfer of IL-13Ralpha2 chain into various solid tumor cell lines that express few IL-13Rs can dramatically sensitize cells to the cytotoxic effect of a recombinant chimeric protein composed of interleukin-13 and a mutated form of Pseudomonas exotoxin A, IL13-PE38QQR. Based on the expression of IL-13R, we have classified five head and neck cancer cell lines into two groups: (a) IL-13Ralpha2 chain-positive cell lines (SCC-25 and KCCT873); and (b) IL-13Ralpha2 chain-negative cell lines (A253, YCUT891, and KCCT871). By plasmid-mediated stable gene transfer, we demonstrate that not only IL-13Ralpha2 chain-positive head and neck cancer cell lines but also IL-13Ralpha2 chain-negative cell lines can dramatically increase sensitivity to IL-13 toxin by 520-1000-fold compared with mock-transfected control cells after genetic alteration to express high levels of the IL-13Ralpha2 chain. In animal studies, i.p. or intratumoral administration of IL13-PE38QQR given daily or on alternate days for 3-5 days showed dramatic tumor response with complete remission in intratumorally injected tumors in both IL-13Ralpha2 chain-positive and -negative but transfected with IL-13Ralpha2 chain head and neck tumor implanted s.c. in nude mice. These results demonstrate that by using a combination approach of gene transfer and systemic

or locoregional cytotoxin therapy, the IL-13R represents a new potent target for head and neck cancer therapy.

Nagao, M., M. Ochiai, et al. (2001). "LacI transgenic animal study: relationships among DNA-adduct levels, mutant frequencies and cancer incidences." *Mutat Res* **477**(1-2): 119-24.

In the processes of carcinogenesis caused by genotoxic carcinogens, DNA-adduct formation and resultant genetic changes are crucially important. In this report, the relationship between DNA-adduct levels and mutant frequencies (MFs), DNA-adduct levels and cancer incidences, and MFs and cancer incidences induced by heterocyclic amines (HCAs), to which humans are exposed on daily basis were investigated. There was no direct correlation between adduct levels and MFs detected after feeding Big Blue mice with 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), in a comparison among various organs. Further, there was no direct correlation between DNA-adduct levels and cancer incidences, in a comparison among various organs of F344 rats. Since DNA-adducts are fixed as mutations after cell proliferation, and mutations in cancer-related genes result in cancer development, it is expected that MFs directly correlate with cancer incidences. However, there was no direct correlation between MFs and cancer incidences. Possible mechanisms involved in the discordance between DNA damage markers and cancer incidences are discussed.

Oshima, M. and M. M. Taketo (2002). "COX selectivity and animal models for colon cancer." *Curr Pharm Des* **8**(12): 1021-34.

Early experiments performed during 1980s and 1990s using carcinogen-induced rat intestinal tumor models demonstrated the inhibitory effects of non-steroidal anti-inflammatory drugs (NSAIDs) on intestinal tumorigenesis. Furthermore, epidemiological studies and clinical trials for familial adenomatous polyposis (FAP) patients supported the possibility that NSAIDs can be used as chemopreventive agents. The major target molecules of NSAIDs are cyclooxygenases (COX), which catalyze the rate-limiting step of prostaglandin biosynthesis. Two isoenzymes of COX have been identified; COX-1 and COX-2. Whereas COX-1 is expressed constitutively in most tissues and responsible for tissue homeostasis, COX-2 is inducible and plays an important role in inflammation and intestinal tumorigenesis. A genetic study using compound mutant mice of COX-2(-)/(-), and Apc(Delta716) which is a model for human familial adenomatous polyposis (FAP), directly demonstrated that induction of COX-2 is critical for intestinal poly

formation. Numerous studies have also demonstrated that COX-2 selective inhibitors suppress intestinal polyp formation in Apc gene-mutant mice, and xenografted cancer cell growths. In addition, stimulation of angiogenesis is one of the major effects by COX-2 expression that is induced in the polyp stromal cells. On the other hand, another study indicated that COX-1 also plays an important role in the early stage of intestinal tumorigenesis. These data from animal model studies should be helpful in understanding the in vivo mechanism(s) of tumor suppression by NSAIDs or COX-2 inhibitors. In this article, Oshima et al we reviewed the animal studies and reported to suppress intestinal tumor growths by NSAIDs or COX-2 inhibitors.

Yi, B., P. J. Williams, et al. (2002). "Tumor-derived platelet-derived growth factor-BB plays a critical role in osteosclerotic bone metastasis in an animal model of human breast cancer." *Cancer Res* **62**(3): 917-23.

Breast cancer produces a variety of growth factors to promote its behavior at primary and secondary sites in autocrine/paracrine manners. However, the role of these growth factors in the colonization of cancer cells in bone, which is one of the most common metastatic sites, is poorly understood. To study this, we established an in vivo model in which the MCF-7 human breast cancer cells caused predominant osteosclerotic bone metastases 20-25 weeks after inoculation into the left cardiac ventricle in female nude mice. To make this model more time efficient, we overexpressed the oncogene Neu, which is associated with aggressive behavior in human breast cancers, in MCF-7 cells (MCF-7/Neu). MCF-7/Neu cells grew without estrogen and developed osteosclerotic bone metastases in 10-12 weeks in animals. Of note, MCF-7/Neu-bearing mice showed substantial plasma levels of human platelet-derived growth factor-BB (hPDGF-BB; 855 +/- 347 pg/ml; mean +/- SE, n = 5), indicating hPDGF-BB production by inoculated MCF-7/Neu cells. MCF-7/Neu cells in culture also produced large amounts of hPDGF-BB. Conditioned medium harvested from MCF-7/Neu cells stimulated osteoblastic bone formation in organ cultures of neonatal mouse calvariae, and a neutralizing antibody to hPDGF-BB blocked the osteoblastic bone formation. Stable transfection of the hPDGF-B AS in MCF-7/Neu cells reduced hPDGF-BB production in culture. Mice bearing these MCF-7/Neu cells with antisense showed reduced bone metastases with decreased plasma hPDGF-BB levels (54 +/- 20 and 35 +/- 21 in two different antisense and 696 +/- 312 pg/ml in empty vector; mean +/- SE; n = 5). Introduction of hPDGF-B cDNA in the MDA-MB-231 human breast cancer cells, which consistently formed osteolytic bone

metastases, induced osteosclerotic lesions in the osteolytic bone metastases. In conclusion, we show that MCF-7 cells cause osteosclerotic bone metastases and that Neu enhances this capacity of MCF-7 cells. Our data suggest that MCF-7/Neu-derived hPDGF-BB plays a causative role in the development of osteosclerotic bone metastases in this model.

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