The role of CDK8、STAT1 and TMEFF2 in colorectal cancer

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Abstract: In the development and progression of cancer, it is estimated that over a million new cases of colorectal cancer (CRC) are diagnosed yearly, accounting for more than 9% of all new cancer cases [Quinn M, et al.2001]. It is also the second most common cause of cancer-related deaths. Despite the recent advances in treating cancer, the 5-year survival rate from CRC remains at 50% and 10% for TNM stages III and IV, respectively[Jonathan A D Simpson,et al.2010]. In this study we investigated the molecular and clinical features of CDK8、STAT1 and TMEFF2-expressing colorectal cancers, the immunohistochemistry was used to detect the expressions of the CDK8、STAT1 and TMEFF2 in these colorectal tissues respectively. The expression of CDK8 locates in nucleus，the positive rates （100 cases colorectal cancer、15 cases adenomas and 15 cases normal mucous） were 37%（37/100)、0(0/15）and 0（0/15）respectively.The CDK8 expression in colorectal cancer was significantly higher than that in normal mucosa and colorectal adenomas (P <0.05); The CDK8 expression had no significant correlation with the clinicopathological factors in patients (P> 0.05)；there was a clear CDK8 protein loss for the older specimens(P＜0.05). The STAT1 expression locates in cytoplasm and nucleus,，and the positive rates were 26%（26/100)、73%(11/15）and 67%（10/15）respectively.The STAT1 expression in colorectal cancer was significantly lower than that in normal mucosa and colorectal adenomas（P＜0.05)；.The STAT1 expression in colon cancer was higher than that in rectal cancer.The TMEFF2 expression were all negative. The CDK8 abnormally high expressed in colorectal cancer,This study suggested that the CDK8 plays a important role in the development colorectal cancer.The decreased expression of STAT1 in colorectal cancer associated with the development of the colorectal cancer; the STAT1 expression was related with tumor location.The loss expression of the TMEFF2 may not be involved in the development of the colorectal cancer.

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Colorectal cancer is a common digestive cancer, its occurrence is a multi-stage, multiple genes process, involving both the activation of multiple oncogenes and tumor suppressor gene inactivation. Currently, there are four treatment options for CRC: surgery, chemo-therapy, radiotherapy and monoclonal antibody therapy. These different modalities can be used alone or in combination, depending on the stage of disease and fitness of the patient. Despite the historical success of these modalities, none of them takes into account the individual tumour biology or the immune response to the cancer.Cyclin dependent kinase 8 (CDK8)，which encodes a member of the mediator complex, is located at13q12.13, a region of recurrent copy number gain in a substantial fraction of colon cancers.It has recently proven to be an effective cancer gene, including colorectal cancer protein and the development of a variety of malignancies,[Firestein R et al.2009];Signal transducers and activators of transcription 1(STAT1) inhibits tumor formation through a negative regulator of cell proliferation and angiogenesis [Klampfer L,et al.2006], the inactivation of the pathological process for colorectal cancer is important; transmembrane protein with EGF-like and two follistatin-like domains 2 (TMEFF2) is very limited in human normal tissues,Recent studies have shown that it did not exhibit the characteristics of tumor suppressor genes [Daniel E.H,et al.2004]. In this study, immunohistochemistry detected CDK8, STAT1 and TMEFF2 proteins in colorectal cancer, colorectal adenoma and colorectal normal mucosa, we analyzed clinical data and pathological features, explored the role of three proteins in colorectal cancer occurrence and development .Understanding the molecular pathology of colorectal cancer may improve therapeutic and diagnostic strategies.our data define CDK8 and STAT1expression in colorectal cancer as a biomarker with potentially important therapeutic implications.

Materials and Methodes

1. Study population

Three hundred and five cases were collectedfrom patients undergoing elective surgical

resection of a histologically proven colorectal cancer, colorectal adenoma and colorectal normal mucosa, The samples were collected between January 2002 and December 2012 from three hospitals,100 colorectal cancer in CDK8 group, 15 colorectal adenoma, 15 normal colorectal tissue;50 men and 50 women in colorectal cancer, aged 21-84 years, mean 64 years, 61 colon cancer, 39 rectal cancer, High and moderately differentiated was 57 cases, Low and undifferentiated was 43 cases,According to TNM staging, Ⅰ, Ⅱ stage was 46 patients, Ⅲ, Ⅳ was 54 cases. 15 casescolorectal adenoma, 15 cases normal colorectal tissue; 52 males and 48 females were colorectal cancer, aged 21 -86 years, mean age 64 years, 62 cases of colon cancer, rectal cancer, 38 cases well-differentiated, 59 cases moderately differentiated, poorly differentiated, undifferentiated 41 cases; Ⅰ, Ⅱ patients with 44 cases, Ⅲ, Ⅳ period of 56 cases,15 colorectal cancer, 15 colorectal adenoma and 15 normal colon mucosa in TMEFF2 group. All specimens received in the histopathology laboratory were incised, fixed immediately in formaldehyde and processed through to embedding in paraffin wax, ensuring optimal tissue fixation and preservation for histological examination. all patients were not receiving chemotherapy， radiotherapy or other treatment before biopsy, pathology diagnosed was definitude after biopsy.Informed consent was obtained from the patients for the use of the resected samples for research. The study complied with the appropriate institutional guidelines.

2. Immunohistochemistry

Tissue sections were placed in a hot oven at 60℃ for 30 min,dewaxed in xylene and then rehydrated in three baths of 100%,90% and 70% ethanol. Endogenous peroxidase activity was blocked using a 0.3% solution of hydrogen peroxide in Tris-buffered saline (TBS). Antigen retrieval was achieved using a rotary microwave oven; the slides were immersed in citrate buffer (pH6.5) and placed in the centre of the oven for 10 min at 800 W then for another 10 min at 300 W. The slides were cooled down immediately for 10 min with tap water,serum in TBS was added to block non-specific adsorption of the antibodies to the tissue.human monoclonal antibodies anti-CDK8 antibody (sigma, USA), anti-STAT1 antibody (sigma, USA) and anti-TMEFF2 antibody (sigma, USA) were incubated with the tissue sections for 1h at room temperature. Universal streptavidin-biotin-peroxidase and DAB kits were used to detect specific antibody binding according to the manufacturer's instructions.The slides were finally counterstained with haematoxylin (sigma, USA), then dehydrated and mounted.Negative controls were done by omitting the primary antibody.

3.Scoring

Positive: CDK8 appears clear tan or brown staining in the background of nucleus; STAT1 appears clear tan or brown stain in the background of the cytoplasm or nucleus; TMEFF2 appears clear background tan or brown stain in skin cells or fibroblasts cytoplasm or the nucleus[3].Accordance with the degree of positive staining cells (antigen content), can be divided into weakly positive (+); middle positive (++); strongly positive (+++). According to the number of positive cells, can be divided into:weak positive (+; the total number of positive cells in 25% or less); middle positive (++; the total number of positive cells in 25% -49%); strongly positive (+++; more than 50% positive cells of the total number). In this study, We used an integrated measurement points. The formula is: (+)% × 1+ (++)% × 2 +(+++)% × 3; total value of <1.0 for the (+) ,1.0-1 .5 were (++),> 1.5 were (+++).least 5-10 HPF were observed.

4 Statistical analysis

Data are expressed as mean ± SD. Differences between groups were compared using Student's t test. Pearson's $\chi 2$ test was used to analyze the correlation between variables. $P<0.05$ was considered to be statistically significant. Data were analyzed using SPSS 12.0.

Results

1. CDK8, STAT1 and TMEFF2 expression

CDK8 expression in colorectal cancer, colorectal adenoma and colorectal normal mucosa were 37% (37/100), 0% (0/15) and 0% (0/15) respectly, CDK8 expression in colorectal cancer tissue was significantly higher than the normal colorectal mucosa and adenomas ($\chi 2 = 6.575$ $p = 0.010$), As shown in Figure 1 A, B, C and Table 1; STAT1 in colorectal cancer, colorectal adenoma and colorectal normal mucosa were 26% (26/100), 67% (10/15) and 73% (11/15) respectly,STAT1 expression in colorectal cancer was significantly lower than normal colorectal tissue ($\chi 2 = 11.310$ $p = 0.001$) and adenoma ($\chi 2 = 8.229$ $p = 0.004$), but there was no significant difference in the expression between the normal mucosa and colorectal adenomas, such as Figure 1 D, E, F and Table1. As shown in Figure G, H,TMEFF2 was negativein in colorectal cancer, colorectal adenoma and colorectal normal mucosa.

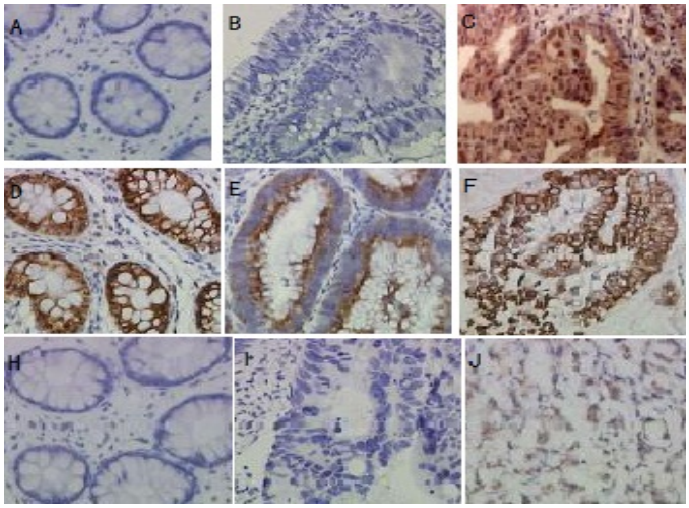

Figure 1. CDK8, STAT1, and TMEFF2 in colorectal carcinoma.
No CDK8 expression in normal colorectal tissue (A) and adenoma (B); visible expression in the cell nucleus of colorectal cancer (C); STAT1 expression in most normal colon tissue (D) and the cytoplasm or nucleus of colorectal adenomas (E), occasionally expressed in colorectal cancer cells (F); No TMEFF2 expression in normal colorectal tissue (H), colorectal cancer (J),visible expression in prostate cancer (J).Evision two-step method, DAB staining × 400.

Table 1. CDK8 and STAT1 expression in each group

pathological features	N	STAT1				N	CDK8			
		positive	negative	x^2	P		positive	negative	x^2	P
Normal mucosa	15	11	4	11. 310	0. 001	15	0	15	6. 575	0. 010
Adenoma	15	10	5	8. 789	0. 003	15	0	15	6. 575	0. 010
Colorectal cancer	100	26	74			100	37	63		

2. Clinical pathological and CDK8, STAT1 expression

STAT1 expression in colorectal cancer was significantly lower than the colon ($\chi 2 = 4.759$ $p = 0.0029$), there was no significant correlation in STAT1 expression (including age, sex, differentiation, stage, whether the lymph nodes transfer) ($p > 0.05$), As shown in Table 2; there was no significant correlation in CDK8 expression (including patient age, sex, tumor location, differentiation, stage, lymph node metastasis).

Table 2. The relationship of CDK8 and STAT1 between the expression and clinicopathological factors

pathological features		STAT1 N	positive	negative	x^2	P	CDK8 N	positive	negative	x^2	P
Age	＜65	44	14	30			45	17	28		
	≥65	56	12	44	1 .382	0 .240	55	20	35	0. 021	0. 884
Sex	M	52	15	37			50	17	33		
	W	48	11	37	0 .456	0. 499	50	20	30	0. 386	0. 534
Location	colon	62	21	41			61	20	41		
	rectum	38	5	33	5. 254	0 .022	39	17	22	1. 191	0. 275
Differ	H/ M	59	18	41			57	20	37		
	L/U	41	8	33	1. 520	0. 218	43	17	26	0. 208	0. 648
Tumor-stage	Ⅰ、Ⅱ	44	13	31			46	17	29		
	Ⅲ、Ⅳ	56	13	43	0. 513	0. 474	54	20	34	0. 000	0. 993
Lymph-metastasis	N	44	13	31			46	17	29		
	Y	56	13	43	0. 513	0. 474	54	20	34	0. 000	0. 993

Discussion

Colorectal cancer is the colorectal epithelium and glandular malignancies,ranking the second cause of cancer deaths in recent years, the incidence was significantly increased.The pathological process of colorectal cancer involved ina variety of molecular biological mechanisms,A variety of oncogenes and tumor suppressor genes were involved in the process of development of cancer from the normal mucosa.CDK8 resides on a region of Chromosome 13 that is known to undergo chromosomal gain in 60% of colorectal cancers[Firestein R,et al.2008;Tsafrir D,et al.2006; Sheffer M,et al.2009;Martin ES, et al.2007].Previous studies have shown that CDK8 is overexpressed in a subset o f colorectal cancers[Firestein R,et al.2008;Tsafrir D,et al.2006;Martin ES, et al.2007],4,28,30.Recently, CDK8 proved to be an effective cancer proteins, including a variety of malignancies, including colorectal cancer incidence and development. there is a typical WNT / β pathway of unusual activity in almost all colorectal cancer, the activity of this pathway was related to colorectal cancer growth, invasion, survival[Conaway,R.C,et al.2005], but the full formation of malignant tumors need other genes participation, such as CDK8. CDK8 regulated the activities of β-catenin gene in colorectal cancer, and play an important role in cancer cell proliferation [Firestein R,et al.2009].In this study, CDK8 expression in colorectal cancer was significantly higher than the normal colon mucosa and colorectal adenomas,so,CDK8 play an important role in the pathological process of colorectal cancer, indicated that CDK8 was the colorectal cancer gene. the high expression of CDK8 in a considerable part of colorectal cancer cases; the expression of 37% was higher than 26% of thef irst results of Firestein [Firestein R,et al.2009] but less than 70%-positive rate as shown in the second [Firestein R,et al.2010]; the results can be powerful description of CDK8 in the high expression rates of colorectal cancer.between CDK8 expression and clinicopathological factors,there were not relationship in age, sex, tumor location, tumor differentiation, tumor stage, lymph node metastasis,CDK8 expression is not correlated with the findings of Firestein, Men CDK8 expression results are slightly different from female colorectal cancer .

STAT1 gene is located on 2q32.2, encoding 750 amino acid residues with a molecular weight of 91kDa, which can be activated by a variety of ligands, including IFNα, IFNγ, EGF, PDGF and IL6. In contrast to other STATs, STAT1 played a negative regulatory role in tumor cell growth[Yu H,et al.2004]. STAT1 negatively regulated the cell proliferation and angiogenesis, thereby inhibiting tumor formation. STAT1 is an important molecule in IFN-γ signaling pathway,endogenous INF-γ and STAT1 form a basic

monitoring system to control the formation of chemically induced and spontaneous tumor development.but it played an important role in the regulation of tumor progression.STAT1 low expression is mainly due to DNA methylation of Colorectal cancer [Dhruva Kumar Mishra,et al.2010] and STAT1 miRNA interference [Gregersen LH,et al.2010].Our study found that STAT1 expression in Colorectal cancer was significantly lower than intestine colorectal adenomas and large intestine normal mucosa,As a tumor suppressor gene，STAT1 which downregulated in colorectal lose its inhibitory role in tumor development, In addition, the absence of STAT1 expression was lower in rectal cancer than colon cancer,In other malignant consistent with the results. indicating the location of the tumor in the rectum or colon, Klampfer [Lidija Klampfer,t al.2008] thought that the STAT1 expression levels significantly decreased in transformed intestinal epithelial cells,In this study,Only the STAT1 expression decreased in colorectal cancer,compared with the STAT1 expression of normal colorectal mucosa,There was not statistically significant in colorectal adenomas; In addition, Simpson et al [Simpson JA,et al.2010] found that nucleus STAT1 was the independent prognostic indicators for colon cancer, compared to with low levels the nucleus STAT1 of rectum Cancer,Survival time of colon cancer patients with high levels of nucleus STAT1 was extension,All of these results also explain that STAT1 played an important role in the development and occurrence of colorectal cancer.

TMEFF2 located in 2q32.3, 246,666 base pairs,17 exons,encoding the transmembrane protein containing 374 amino acid, it was tissue-specific expression,its expression is very limited in normal tissue, mainly expressed in the prostate and central nervous system, in the colon tissue,only expressed TMEFF2 mRNA and without protein express [Young J,et al.2001]. TMEFF2 in prostate cancer was over-expression [Daniel E.H,et al.2004], people thought that TMEFF2 acted as a tumor suppressor gene, TMEFF2 may inhibit tumor cell growth, prostate cancer showed a higher degree of malignancy without TMEFF2 expression;But Afar [Daniel E.H,et al.2004] proved that TMEFF2 did not show the characteristics of tumor suppressor genes, overexpressing TMEFF2 did not inhibited cell proliferation in prostate cancer PC3 cell line. In addition, there is a wide range of TMEFF2 methylation [Qiong He,et al.2010] and the phenomenon of frequently loss of heterozygosity in colorectal cancer [Fabian Model,et al.2007], so that the TMEFF2 mRNA had no expression in the majority of colon tumors and other tumors, TMEFF2 can not act as a tumor suppressor gene. In this study, TMEFF2 protein expression can be detected in prostate cancer,However, TMEFF2 protein expression can not be detected in 15 cases of colorectal cancer, 15 cases of colorectal adenomas and 15 normal colorectal mucosa tissues, our results further confirmed that there was no TMEFF2 protein in the large intestine, TMEFF2 was tissue-specific expression; the same time, we provided further evidence for the questioned whether TMEFF2 was the tumor suppressor gene, at least, TMEFF2 did not expresse tumor suppressor protein in colorectal cancer.we must recognized that there was TMEFF2 mRNA expression, but no protein product in the normal colon tissue, and did not shows no methylation in most of the normal colon and inflammatory bowel disease tissue, the imechanism needs further study.

In summary, we have conducted a large sample of CDK8, STAT1, TMEFF2 express.Our findings show that CDK8 is expressed in a high fraction of colorectal cancers,over-expression CDK8 played an important role in the development of colorectal cancer; STAT1 were down-regulated in colorectal cancer, the absence of STAT1 expression was related to the occurrence and development of colorectal cancer;compared to rectum cancer,the absence of STAT1 expression is more obvious than the colon; there was no TMEFF2 protein expression in colorectal tissue, TMEFF2 is not the tumor suppressor gene of colorectal cancer. Thus, CDK8 and STAT1 may be the new indication and new therapeutic targets for the detection of colorectal cancer.these findings may be of great use in defining patients that may be distinctly susceptible to small molecule-based therapies.

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