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Full Length Research Paper

Expression of retinoic acid receptor β in human colorectal cancer

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To investigate the expression of retinoic acid receptor β (RAR-β) in human colorectal cancer, RAR-β was detected by immunohistochemistry in normal, adjacent and tumor colorectal tissues of 60 patients suffering from colorectal cancer. The clinicopathological data of these 60 patients were also collected and analyzed. The expression rate of RAR-β in tumor tissues was significantly lower than those of both normal and adjacent tissues. It was also lower in patients with lymph node metastasis and in advanced stage than those without metastasis and in early stage. The expression rate of RAR-β decreases in human colorectal tumor tissues, suggesting RAR-β may be involved in the formation of human colorectal cancer. RAR-β may become a new prognostic indicator of human colorectal cancer.

Key words: Colorectal neoplasms, retinoic acid receptors, immunohistochemistry.

INTRODUCTION

In recent years, the incidence of human colorectal cancer in China has increased dramatically, and its prevention has become a research hotspot. Retinoic acid receptor β (RAR-β) is the most attractive retinoic acid receptor subtype. Accumulating studies have found that the expression of RAR-β is usually silenced in various types of human tumor tissues, which has led to the hypothesis that RAR-β may act as a tumor suppressor (Therapy, 2004). However, the status of RAR-β in colorectal cancer has only been examined in cell lines but not in gross specimen yet.

In the present study, we detected the expression level of RAR-β in normal, adjacent and tumor tissues of 60 cases of colorectal cancer in our hospital by immune-histochemistry. These data, together with the clinic-pathological data of these 60 patients, were analyzed to examine the expression of RAR-β in human colorectal cancer and its potential clinical significance.

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Abbreviations: RAR-β, Retinoic acid receptor β; **TNM,** tumor, node, metastasis; **S-ABC,** strept avidin biotin-peroxidase complex; **PBS,** phosphate buffered saline; **RXRs,** retinoid X receptors.

MATERIALS AND METHODS

Clinical data

Sixty (60) patients who accepted surgical treatment of colorectal cancer from 2006 to 2007 in the third affiliated hospital of Sun Yet-Sen University were selected, including 31 males and 29 females, aged from 18 to 89 years with an average age of 59.8 years. None of them accepted radiotherapy and chemotherapy preoperatively. Gross specimens were confirmed to be colorectal cancer by pathology. The 60 cases of tumor tissues included 3 cases of well differentiated, 43 cases of middle differentiated and 14 cases of poorly differentiated. According to the tumor, node, metastasis (TNM) staging system (2002 UICC, 6th edition), there were 9 cases of I stage, 20 cases of II stage, 20 cases of III stage and 11 cases of IV stage. Normal (5cm away from tumor tissues), adjacent (3 cm away from tumor tissues) and tumor tissues of paraffin sections were made.

Main reagents and instruments

Anti-RAR-beta, a rabbit polyclonal antibody and Streptavidin

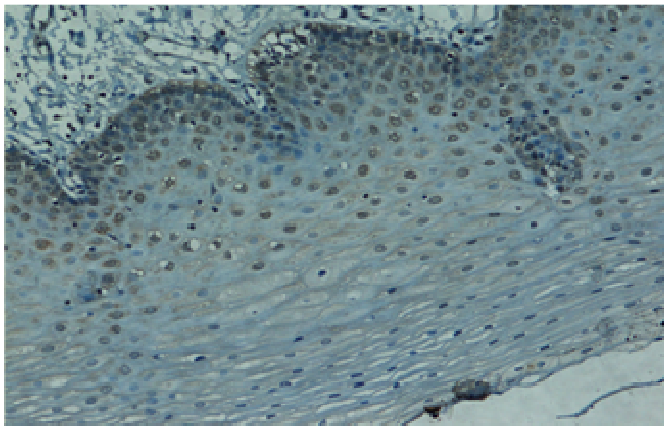

Figure 1. Expression of RAR-β in normal esophageal tissues, S-ABC method, x200.

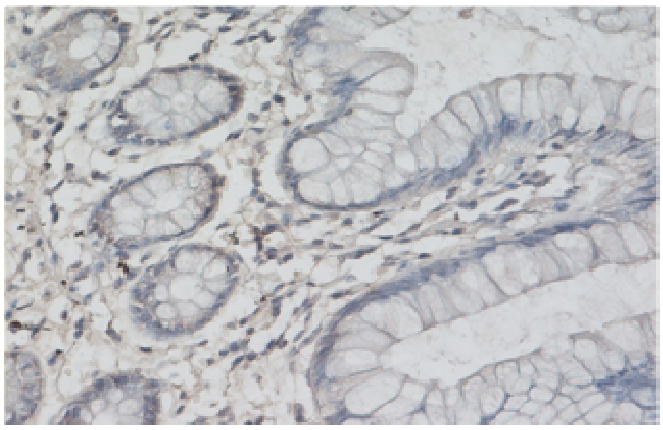

Figure 2. Negative control of normal colorectal tissues, S-ABC method, x400.

peroxidase immunohistochemistry kit, were purchased from Beijing Biosynthesis Biotechnology Company (E-mail: bioss@bioss.com.cn). Fluorescence microscope was supplied by Nikon Company.

Materials

Sixty (60) cases of gross specimens, including tumor, adjacent and normal tissues, were processed routinely (10% formalin fixed for 24 – 48 h), paraffin-embedded and thin sectioned (4 μm).

Strept avidin biotin-peroxidase complex (S-ABC) immunohistochemistry method

The dilution of RAR-β was 1:300. Phosphate buffered saline (PBS) solution was used as negative control, and slides of normal esophagus tissues were used as positive controls.

Scoring of immunohistochemistry

Evaluation of RAR was carried out in high-power fields (×400) using a standard light microscope. Two doctors simultaneously searched the entire tissue section and determined the most representative areas using a double-headed microscope. After completely reviewing the immunostained sections of each lesion, a total of 500 cells from five different representative fields were counted independently. Cases with discordant results (interobserver differences of more than 5%) were reevaluated. Brown granules located in the nuclear indicated positive expressions. Less than 10% of cells with positive expressions was the standard for negative, 10 - 30% for positive (+), 30 - 50% for medium positive (++) and more than 50% for strong positive (+++).

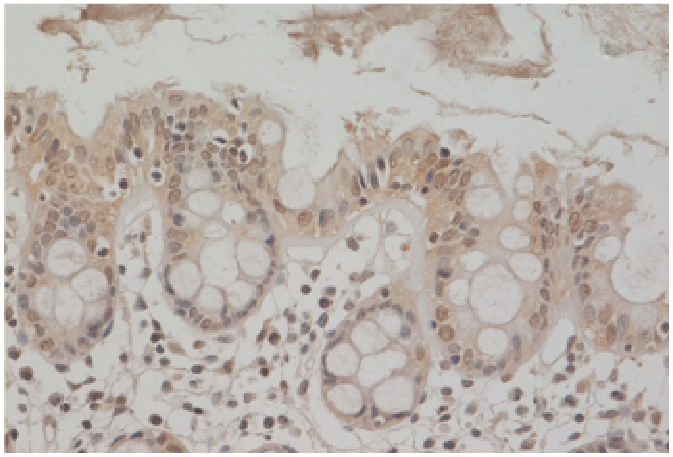

Figure 3. Expression of RAR-β in normal colorectal tissues, S-ABC method, x 400.

Staging of clinicopathological data

TNM staging system of colorectal cancer was used.

Statistical analysis

The expression rates of RAR-β in tumor, adjacent and normal tissues were calculated and compared with rank sum test and McNemar two-related-samples test. The relationship between RAR-β and clinicopathological data was calculated and compared with rank sum test and Spearman correlational analysis method. All the results were analyzed by the statistical package for the social sciences (SPSS) 11.0 soft and P-values less than 0.05 were considered significant.

RESULTS

Expression of RAR-β in tumor, adjacent and normal tissues of 60 patients

The expressions of RAR-β in 10 cases of normal esophagus tissues were strong positive (Figure 1). Negative control of normal colorectal tissues showed negative expression of RAR-β (Figure 2). The expression rate of RAR-β in tumor tissues was significantly lower than those of both normal and adjacent tissues ($P = 0.000 < 0.01$), while there was no difference between the adjacent and normal tissues (Figures 3 – 5 and Table 1).

Table 1: Expression of RAR-β in tumor, adjacent and normal tissues of 60 patients

Tissues	Cases	Expression of RAR-β					
		-	+	++	+++	Positive rate	P value
Tumor	60	31	27	2	0	48.3%	P=0.000<0.01*
Adjacent	60	10	25	23	2	86.7%	
Normal	60	10	22	25	3	86.7%	

*the differences between tumor and normal tissues, adjacent and normal tissues were significant (P=0.000<0.01).

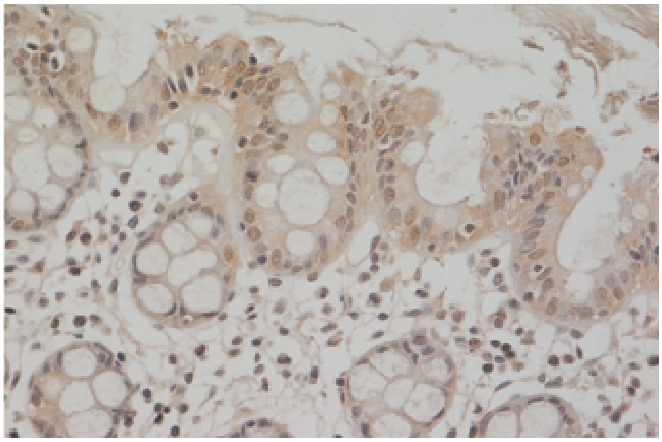

Figure 4. Expression of RAR-β in adjacent colorectal tissues, S-ABC method, x400.

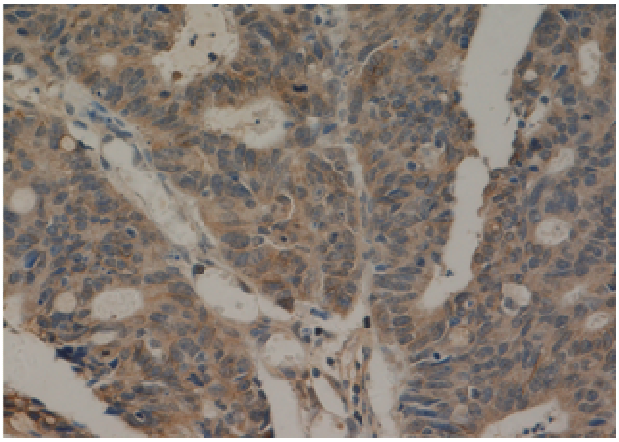

Figure 5. Expression of RAR-β in colorectal tumor tissues, S-ABC method, x 400.

The relationship between RAR-β and clinico-pathological data

The expression of RAR-β in tumor tissues had no correlation with sex, age or differentiation degree of tumor. The expression rate of RAR-β was lower in tumor tissues of patients with lymph node metastasis (32%) than those without metastasis (60%) (P = 0.033 < 0.05). Meanwhile, it was lower in tumor tissues of patients in advanced stage (stage III and IV of TNM stage) (29.0%) than those in early stage (stage I and II) (69.0%) (P = 0.002 < 0.01) (Table 2).

DISCUSSION

With the improvement in living condition, the incidence and mortality rate of colorectal cancer is rising gradually in China. There were 150,656 cases of colorectal cancer and 89,102 death cases in 2002. The 5-year-incidence is the 3rd place of tumors in China (http://www-depdh.iarc.fr/globocan/GLOBOframe.htm). So, colorectal cancer has been ranked as one of the eight malignant tumors that require special prevention and treatment in China (Bulletin of Chinese Cancer, 2004).

In recent years, one of the most important breakthroughs in human colorectal cancer research is the identification of nuclear retinoid receptor as a potential target in treating human colorectal cancer. Researches have found that nuclear retinoid receptors can be divided into retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Both RARs and RXRs have three subtypes (α, β and γ). RAR-β has attracted more attention because it has been considered as a putative tumor suppressor gene primarily due to its frequent loss of expression in various types of human cancers including lung cancer, oral cancer, breast cancer, esophageal cancer, prostate cancer, bladder cancer, endometrial cancer, etc (Xiaoqiang et al., 2002; Lotan et al., 1995; Wang et al., 2005; Qiu et al., 1999; Nakayama et al., 2001; Mingyi, 2008; Kojiro et al., 2008).

The status of RAR-β in colorectal cancer has only been

Table 2. The relationship between RAR-β and clinicopathological data.

Clinicopathological data		Cases	Expression of RAR-β		P value
			-	+~+++	
Sex	Male	31	16	15	P = 0.800 > 0.05
	Female	29	15	14	
Age	≥60 y	34	19	15	P = 0.463 > 0.05
	<60 y	26	12	14	
Lymph nodes metastasis	Yes	25	17	8	P = 0.033 < 0.05
	No	35	14	21	
Differentiation degree of tumor	Well	3	0	3	P = 0.646 > 0.05*
	Middle	43	23	20	
	Poor	14	8	6	
TNM pathological stage	I	9	3	6	P = 0.002 < 0.01#
	II	20	6	14	
	III	20	13	7	
	IV	11	9	2	

*P value was calculated by comparison of well + middle groups and poor group. # P value was calculated by comparison of stage I + II groups and stage III + IV groups.

examined in cell lines (Shi-Yong, 2004) but not yet in gross specimen. Our research showed that the expression rate of RAR-β decreased dramatically in tumor tissues of colorectal cancer and the decreasing degree was related to the metastasis situation of lymph nodes and pathological stage of tumors, suggesting that the loss of RAR-β might be the key progress of colorectal carcinogenesis. So, detection of RAR-β in tumor tissues might be an effective method to evaluate the prognosis of patients with colorectal cancer postoperatively.

Our research also found that the expression of RAR-β had no correlation with the differentiation degree of tumors, which was not consistent with some studies on other tumors (Kojiro et al., 2008). The reason might lie in the insufficient cases of well differentiation tumor tissues and we might select more cases to determine the relationship between the expression of RAR-β and differentiation degree of tumor next time. The retinoids (vitamin A and its biologically active derivatives) are hotspots in cancer treatment and chemoprevention. Previous reports have shown that the retinoid-mediated growth inhibition of colorectal cancer cell lines can be reduced by the knockout of RAR-β gene or blockage of its expression, and inhibition of retinoid-resistant cell lines can be induced by restoration of RAR-β expression (Lee et al., 2000; Nicke et al., 1999; Stewart et al., 1997), which suggests the importance of RAR-β in retinoid-mediated chemotherapy.

Therefore, whether it is necessary to detect the expression of RAR-β in colorectal tumor tissues for evaluating the sensitivity of patients to retinoid-mediated chemotherapy or not may be another hotspot in the future.

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