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EXPRESSION OF TGF-β_1 AND TGF-β R II IN COLORECTAL ADENOMAS OF VARYING STAGES

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Abstract: To study the function of transforming growth factor β_1 (TGF-β_1) and transforming growth factor-β type II receptor (TGF-βR II) in varying stages of colorectal adenoma during neoplastic transformation, fifty-five cases of resected colorectal adenomas were examined immunohistochemically. Our results showed that the expression of TGF-β_1 ranged from high to low, with the progression of low-grade to high-grade dysplasia of adenomas to carcinoma. And there was significantly different expression of TGF-β_1 between moderate and high dysplasia adenomas ($P < 0.05$), but no significantly different expression of TGF-β R II was found among varying stages of adenomas. Our results suggested that the quantities of TGF-β_1 secreted by adenoma cells decrease dramatically in moderate to severe dysplasia colorectal adenomas. It is the decreased secretion of TGF-β_1, rather than the mutated TGF-βR II that may play an important role in transforming colorectal adenomas to adenocarcinomas.

Key words: colorectal adenomas, TGF-β_1, TGF-βR II, immunohistochemistry
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INTRODUCTION

Transforming growth factor β_1 (TGF-β_1), a multifunctional cytokine, is reported as a regulator of cell growth and differentiation (Lahm et al., 1993). TGF-β_1 can act as a growth inhibition factor in normal colon cells. Without the regulation of TGF-β_1, the normal colonic epithelium would undergo neoplastic transformation. Transforming growth factor-β type II receptor (TGF-βR II) is a signal transductive molecule, and TGF-βR II gene is a tumor suppressor gene (Brattain et al., 1996). Mutated TGF-βR II gene can inactivate TGF-βR II receptors. Then TGF-β_1 loses its negative regulation effect, and colorectal neoplasms will develop. The role of TGF-β_1 and TGF-βR II in colorectal oncogenesis remains unclear. In order to gain understanding of the possible role of TGF-β1 and TGF-βR II in colorectal oncogenesis, we investigated the expression of TGF-β_1 and TGF-βR II in fifty-five cases of colorectal adenomas by the immunohistochemical method.

MATERIALS AND METHODS

1. Adenoma specimens

Fifty-five January 1993 to March 1998 cases of colorectal adenomas were collected from the Department of Proctology, First Affiliated Hospital, Medical College of Zhejiang University. Materials for this study were obtained from partial colectomy or polypectomy specimens, and consisted of 36 rectum, 11 left colon, 3 transverse colon and 5 right colon. There were adenoma specimens from 32 males and 23 females with age ranging from 25 to 85 years (mean of 60 years).

Excised tissues were fixed in formalin solution and then embedded in paraffin. The adenomas were examined by an experienced pathologist to further confirm the diagnosis. According to the criteria of "National Pathological Research of Colorectal Carcinoma", dysplasia of adenomas are graded as mild, moderate and severe. Fifty of the specimens had regions of carcinoma.

2. Immunohistochemistry

A standard Envision technique was employed using polyclonal antibodies against TGF-β_1 and TGF-βR II respectively. The TGF-β_1 antibody (sc - 146) was an affinity-purified rabbit polyclonal antibody raised against a peptide mapping at the carboxy terminus of the precursor form of TGF-β_1 purchased from Santa Cruz Biotechnology. The TGF-βR II antibody (L - 21) was an affinity-purified rabbit polyclonal antibody raised against the epitope mapping within an internal domain of the TGF-βR II precursor purchased from Santa Cruz Biotechnology. Envision Kit was obtained from DAKO Company. Immunohistochemistry analysis was routinely performed. In each sample, a negative control was examined after the primary antibody was replaced by PBS solution.

In each section, the expression of TGF-β_1 and TGF-βR II was estimated in areas of mild, moderate, severe dysplasia and carcinoma, respectively. The cytoplasm stained with yellow or brown was regarded as positive. The importance of the staining was evaluated by counting the frequency of labeled cells in five high-power magnification fields each containing about 100 cells. The results of immunostaining of TGF-β_1 and TGF-βR II were semiquantitatively estimated as follows: - , staining in 0 ~ 5% of cells; + , staining in 6% - 20% of cells; + + , staining in 21% - 50% of cells(+ and + + were considered to be weak antigen expressions); + + + , staining in 51% - 80% of cells; + + + + , staining in 81% - 100% of cells(+ + + and + + + + were considered to be strong antigen expressions). Correlation between TGF-β_1 or TGF-βR II expression and the different dysplasia of colorectal adenomas were achieved using the Rank test.

RESULTS

1. Expressions of TGF-β_1

The distribution of TGF-β_1 immunostaining are summarized in Table 1.

Strong positive staining was present in most adenomas with mild or moderate dysplasia(Fig. 1). But most severe dysplasia or carcinoma regions of the sections showed negative or weak antigen expression (Fig. 2). The expression of TGF-β_1 decreased in the process of progression from low-grade to high-grade dyaplasia of adenoma and to carcinoma. There was significant difference in the expression of TGF-β_1 among varying stages of adenomas ($P < 0.005$). And the expression of TGF-β_1 in mild and moderate dysplasia adenomas was significantly higher than that in severe dysplastic adenomas and carcinoma regions ($P < 0.05$). But, no difference was observed between mild and moderate dysplasia (or between severe dysplasia and carcinoma) region ($P > 0.05$).

Table 1 Immunohistochemical localization of TGF-β_1 in different areas of colorectal adenomas.

Expression of TGF-β_1	Grade of dyaplasia			Carcinoma	Total
	Mild	Moderate	Severe		
- -	3	4	6	22	35
+	1	2	16	10	29
+ +	4	9	15	7	35
+ + +	2	16	7	3	28
+ + + +	27	21	10	8	66
Total	37	52	54	50	193

3. Expressions of TGF-βR II

The distributions of TGF-βR II immunostaining are summarized in Table 2. Results showed that mild, moderate, severe dysplasia adenomas and carcinomas could not be distinguished on the basis of TGF-βR II staining ($P > 0.05$).

Table 2 Immunohistochemical localization of TGF-βR II in different areas of colorectal adenomas.

Expression of TGF-β R II	Grade of dyaplasia			Carcinoma	Total
	Mild	Moderate	Severe		
- -	16	16	22	24	78
+	6	10	11	9	36
+ +	4	10	7	6	27
+ + +	9	13	12	7	41
+ + + +	4	4	3	3	14
Total	39	53	55	49	196

DISCUSSION

Most colorectal carcinomas are thought to be developped from premalignant adenomatous pol-

yps, in what is called the adenoma – carcinoma sequence (Muto et al., 1975). There is a multistep progression from normal colonic epithelium to adenocarcinoma. Primary studies showed that the activation of proto-oncogenes and mutation of tumor suppressor genes, and also the regulation of growth factors, may play important roles in colorectal oncogenesis. TGF-β_1, a member of the TGF-β family, was reported as a negative regulator of epithelial cells. The main biological function of TGF-β_1 is inhibiting epithelial cell proliferation, including cancer cells. Colorectal neoplasms may result from an imbalance in the production of TGF-β_1 and/or a loss or reduced response to negative regulations of TGF-β_1.

The levels of TGF-β_1 in patients with colorectal cancer were investigated in a previous study (Tsushima et al., 1996). It is considered that plasma TGF-β_1 levels in patients with colorectal cancer or expression of TGF-β_1 in colorectal cancer tissues are significantly higher than those in normal control, and are associated with disease progression. Zhu et al, reported that the expression of TGF-β_1 progressively decreased in normal colonic mucosa, mucosa adjacent to the tumors, and cancer tissues (Zhu et al., 1996). These contradictory results suggest that the role of TGF-β_1 in colorectal carcinoma remains unclear.

However, the expression of TGF-β_1 in the presumed precursors of most colorectal adenocarcinomas has not been extensively documented. Laethem et al, reported the localization of TGF-β_1 precursor in colorectal adenomas and found that the TGF-β_1-LAP expression in epithelial cells did not correlate with the grade of dysplasia (Laethem et al., 1996). Our study indicates that the expression of TGF-β_1 decreases markedly from moderate to severe dysplasia. Because TGF-β_1 plays its role by the autocrine pathway (Coffey et al., 1987), our results also imply that the secretion of TGF-β_1 by colorectal adenomas decrease with progression of adenoma dysplasia, and that the secretion of TGF-β_1 decreases remarkably during the process of adenoma progression from moderate to severe dyaplasia.

The effect of TGF-β_1 on adenomas in vitro was reported by Manning et al (Manning et al., 1991). Their study showed that the conversion of the non-tumorigenic phenotype of human colonic adenoma cell lines to the tumorigenic phenotype was accompanied by a reduced response to the growth inhibiting effects of TGF-β_1. Furthermore, the cell lines used in that experiment were relatively late-stage adenomas. These studies therefore indicated that late-stage adenomas were still responsive to the growth-inhibitory effects of TGF-β_1 and that loss of response to the TGF-β_1 occurred at a relatively late stage in colorectal carcinogenesis. Our study showed that expression of TGF-β_1 reduces with the progression of adenomas. This suggests that the decreased secretion of TGF-β_1, not the reduced response to negative regulation of growth, might play an important role in the process of colorectal adenoma transformation to adenocarcinoma.

The negative regulator effects of TGF-β_1 are initiated following ligand binding to TGF-β type II receptors, then the complex binds to typeI receptor and activates it. If one of the receptors is absent or inactivated, the cells would lose their responsiveness to TGF-β_1, and colorectal neoplasms might develop. TGF-βR II gene is considered to be a novel tumor suppressor gene (Brattain et al., 1996), and plays an important role in colorectal oncogenesis. Several studies showed down-regulation of TGF-βR II in human colorectal cancer (Matsushita et al., 1999; Guan et al., 1998). It indicates that TGF-βtype II receptor is associated with the signal transduction of TGF-β_1 and may play a corresponding role in development of colorectal cancinomas. Eskinazi et al, reported the overexpression of TGF-βR II in sporadic adenocarcinomas, and the existence of a relationship between the abundance of typeII receptor expression and the degree of differentiation of the tumor (Eskinazi et al., 1998). So sporadic colorectal cancers do not necessarily indicate absence or presence of mutated TGF-βR II receptors that could explain a resistance to TGF-β mediated growth inhibition. Although the TGF-βR II gene is generally regarded as a tumor suppressor gene, not all of the colorectal neoplasms are associated with the absence of TGF-βR II, whose effects on the development of colorectal neoplasms remain unclear.

Our study shows no different expression of TGF-βR II among mild, moderate, severe dysplasia adenomas and carcinomas. This suggests

TGF-βR II alterations were not so important for colorectal oncogenesis.

In summary, our findings showed that the TGF-β_1 amount secreted by adenoma cells decrease dramatically from moderate to severe dysplasia colorectal adenomas; and that the decreased secretion of TGF-β_1 may lead to the neoplastic transformation of colorectal adenomas. So, expression level of TGF-β_1 can be used to distinguish early or late – stage adenomas. Inactivation of the TGF-βR II receptor may not be an important event in the process of colorectal oncogenesis. The mechanisms of TGF-β_1 and TGF-βR IIin oncogenesis must be elucidated by further investigation.

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