The Value of p21WAF1 p53 Protein and DNA Content in Large Intestine Cancer

Zhe Li[1], Zhaoyang Li[2], ChangLe Ruan[3]

[1] Colorectal Surgery Department of Taian Cental Hospital, Taian, Shandong, 271000, P.R. CHINA
[2] ShanDong University, Jinan, 250012, P.R. CHINA
[3] General Surgery Department of Qianfo Hospital, Jinan, 250014, P.R. CHINA

Abstract **Objective** To study the role of p21WAF1 and p53 protein in the occurrence and development of large intestine cancer. **Methods** p21WAF1 and p53 protein were examined by immunohistochemistry SP in 41 specimens of large intestine carcinoma and 10 normal specimens. DNA content of corresponding specimen were examined by flow cytometry. **Results** The expression of S phase fraction(SPF), p53 gradually increased among normal mucosa, well differentiated adenocarcinoma, moderately differentiated adenocarcinoma, poorly differentiated adenocarcinoma, while the expression of p21WAF1 gradually decreased in above groups. The expression of p21WAF1 in large intestine cancer of Dukes A+B phase was much higher than that in large intestine cancer of Dukes C+D stages, while the expression of p53 in large intestine cancer of Dukes A+B stages was much lower than that in in large intestine cancer of Dukes C+D stages. **Conclusion** p21WAF1 and p53 played an important role in tumorigenesis and development of large intestine adenocarcinoma.

Key Words p21WAF1; p53; DNA content; colorectal neoplasms

Nowdays, most reports about p21WAF1 and p53 protein in colorectal neoplasms were focused on cell cycle control. Few of them discussed the relation between the two kinds of proteins and clinical data. No reports about the relation between them and DNA content were found. Our research adopted immunohistochemistry to check $p21^{WAF1}$ and p53 protein in 41 specimen of large intestine carcinoma and 10 normal specimen. At the same time, we checked DNA content of the corresponding specimen by flow cytometry.

MATERIALS AND METHODS

Materials

41 cases colorectal adenocarcinoma specimens and 10 cases normal mucosa specimens were obtained from 41 patients who underwent surgical treatment from July 2002 to January 2003 in Qian–fo Hospital, Jinan, P.R. China. There were 23 males and 18 females with a mean age of 55.6 years old (ranging from 28 to 80 years old). There were 19 colon carcinomas and 22 rectum carcinomas with 8 Dukes stage A, 12 Dukes stage B, 10 Dukes stage C, 11 Dukes stage D. All the specimens were divided into two groups. One was keet in refrigerator(–80℃), to other was fixed with 10% formalin, embeded in paraffin and cut at a thickness of four micrometer.

Methods

Main reagent The rat anti –p53 and p21WAF1 monoclonal antibodies were purchased from the Zhongshan Biotechnology Company.Envision TM were from the Dako Company.

Immunohistochemistry The samples were used for SP (strepatavidin peroxidase) immunostaining. Sections were dewaxed, rehydrated and incubated with 0.3% hydrogen peroxide to quench endogenous peroxidase activity. Antigen retrieval was performed by microwave pre –treatmen in 0.01M sodium citrate buffer (PH 6.0) for 10 min at 750 watt. Subsquently, sections were incubated overnigh at 4℃ with monoclonal mouse antibody against p53 (diluted at 1:60), p21WAF1 (diluted at 1:30) respectively. The bound antibody was detected by a streptavidin –biotin–peroxidase complex and visualized by 3,3–dinminobenzidine tetrahydrochloride supplemented with 0.01% hydrogen peroxide. Finally, the slides were lightly counterstained with Mayer´s hematoxylin.All series include positive controls, and omission of the primary antibodies served as negative control. All

Correspondence: Zhe Li, Colorectal Surgery Department of Taian Cental Hospital, Taian, 271000, P.R. CHINA
Email:lizhe700303@yahoo.com.cn

controls gave satisfactory results. Positive signals were shown in nucleus. If there were more than 25% positive cells in cancer sections, the results were regarded as positive, or else negative.

Flow cytometry

The FCM was automatically adjusted at first by using CaliBR ITE beads and the specific software FACSCOMP. And, because of the diversity of scatter mode and autofluo–rescence, each specimen was adjusted before analysis and settings of equipment were optimized for spectral over lap when the samples are stained with the fluo–rochromes. Then DNA content, including S phage fraction(SPF), was determined by flow cytometry.

Statistical Analysis

Statistical Analysis was by Pearson method, student´s test or Chisquane test by means of SPSS10.0 software. For all ststistical analyses, the value of $p<0.05$ was considered to be significant.

RESULTS

The expression of p21WAF1, p53 and SPF had no significant difference respectively in patients with different age, sex and the location of tumor. ($p>0.05$)The SPF and p53 gradually increased among normal mucosa, well differentiated adenocarcinoma, moderately differentiated adenocarcinoma, poorly differentiated adenocarcinoma($p<0.05$), while the expression of p21 WAF1 gradually decreased in above groups($p<0.05$). The SPF and p53 in Dukes A+ B phase of large intestine adenocarcinoma was much lower than those in Dukes C+D stages ($p<0.05$), while the expression of p21WAF1 in large intestine adenocarcinoma of Dukes A+B stages is much higher than that in large intestine adenocarcinoma of Dukes C+D ($p>0.05$) stages.

The expression rate of p53(+) in large intestine adenocarcinoma was 68.2%, and the expression rate of p21WAF1 (+) was 36.6%. The expression of p21WAF1 and p53 had negative corelation ($p<0.05$). The SPF in large intestine adenocarcinoma which expressed both P53(–) and p21WAF1(+) was much lower than those in large intestine adenocarcinoma which expressed both p53(+) and p21WAF1 (–)($p<0.05$).

DISCUSSION

Large intestine cancer was one of the most common neoplasms in China. In recent twenty years, its incidence increased gradually. The mechanism was very complex. Since El–Deiry et al discovered WAF1 gene in 1993 [1], scientists had found that p21WAF1 and p53 protein played an important role in the occurance of large intestine carcinoma.

Table 1 Relations between $p21^{WAF1}$, p53, SPF and clinical features of colorectal cancer

Clinical features		N	SPF(%)	p53(+)		p21WAF1(+)	
				N	%	N	%
sex	male	23	15.63±4.19	16	69.57	9	39.13
	female	18	14.79±4.98	12	66.27	6	33.33
age	≤50	19	16.01±5.09	13	68.42	7	36.84
	>50	22	14.97±4.98	15	68.19	8	36.37
location	left large intestine	29	15.63±5.62	19	65.52	10	34.49
	right large intestine	12	14.98±6.01	9	75.00	5	41.67
DukesA+B stages		20	11.99±3.19	10	50.00	11	55.00
DukesC+D stages		21	17.94±5.58	18	85.71	4	19.05
normal mucosa		10	6.05±2.48	0	0.00	10	100.00
well differentiated adenocarcinoma		8	10.29±2.38	1	12.50	6	75.00
moderately differentiated adenocarcinoma,		26	14.27±2.91	26	80.77	8	30.77
poorly differentiated adenocarcinoma		7	23.31±7.26	6	85.71	1	14.29

Table 2 Relations of p21WAF1, p53 and SPF

		p21WAF1			
		(−)		(+)	
		SPF(%)	N	SPF(%)	N
p53	(−)	14.32	1	12.75±3.23	12
	(+)	18.55±6.07	25	15.23±5.12	3

Wild type p53 was a key gene in G1 check−point. When it was activated, it would transcribe WAF1. P21WAF1 could combine many kinds of Cdk−Cyclin complex and restrained the activaty of Cdk and proliferating cell nuclear antigen(PCNA)[2]. The result would lead to cell cycle arrest in G1 phase and apoptosis, which would provide enough time to repair DNA. This important function in the tumorigenesis and development of large intestine were also confirmed by many scientists.

This study found that the expression of SPF, p53 gradually increased among normal mucosa, well differentiated adenocarcinoma, moderately differentiated adenocarcinoma, poorly differentiated adenocarcinoma, while the expression of p21WAF1 gradually decreased. We also found tnat the expression of p21WAF1 in large intestine cancer of Dukes A+B phase was much higher than that in large intestine cancer of Dukes C+D phase, while the expression of p53 in large intestine cancer of Dukes A+B phase was much lower than that in large intestine cancer of Dukes C+D phase. The results were according to the reports by Doglioni C[3], Viale G[4], Jackson PA[5].

So we could confer that p21WAF1, p53 and DNA content were useful in evaluating prognosis of large intestine adenocarcinoma. They would be applied more and more in diagnosis and gene therapy in the future.

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