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EXPRESSION OF p53 PROTEIN IN CANCERS OF SMALL INTESTINE AND ITS RELATIONSHIP TO CLINICAL COURSE AND PROGNOSIS

Ge Chunlin 葛春林 He Sanguang 何三光 Tian Yulin 田雨霖
Qu Hengchun* 曲恒春 He Anguang* 何安光

*Department of General Surgery, *Institute of Cancer Research, First Affiliated Hospital, China Medical University, Shenyang 110001*

In order to study the relationship of p53 gene mutation with the occurrence and prognosis of cancer of small intestine, expression of p53 protein was immunohistochemically examined. The results showed that p53 protein expression was high in 75% of small intestine cancer, and positive in 21.1% of the tissues close to cancer. In 7 cases of small intestinal adenoma, only one was immunoreactive. Sixteen samples of normal tissue of the intestine didn't show expression of p53 protein. The study also found that the degree of p53 protein expression was significantly correlated with that of tumor cell differentiation, invasion, metastasis and prognosis.

Key words: Duodenal neoplasms, Jejunal neoplasms, Ileal neoplasms, Protein p53.

Wild-type p53 gene is one of the tumor suppressor genes, it promotes limitless proliferation of cells when having mutation or deletion, and it is one of the factors for tumor's extremely active proliferation, playing an important role in tumor's occurrence and development. By detecting p53 protein expression in small bowel cancer, we tried to demonstrate the relationship between p53 gene mutation and intestinal cancer's occurrence, development and prognosis.

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MATERIALS AND METHODS

Clinical Data

Seventy-two cases of small bowel cancer (51 duodenal cancers, 9 jejunal cancers, 12 ileal cancers), 19 cases of tissue close to cancer, 6 cases of metastases lymph node and the liver, 7 cases of intestinal adenoma, 16 cases of normal intestinal tissue. The above samples were taken from patients undertaking surgical operation from 1975 to 1994 in the First and Second Affiliated Hospital of China Medical University. All samples were 10% formalin-fixed and paraffin-embedded. Among 72 cases of small bowel cancer, 47 were male and 25 were female, the age ranged from 17 to 83 years with the average of 48 years old. 8 received exploratory laparotomy or gastroenterostomy or cholecystoenterostomy, 64 received radical resection. Among the 64 cases, 2 died in hospital, the other 62 cases were followed-up after operation till dec. 31, 1994. 57 cases were successfully followed up, and 5 cases were lost, the following rate was 91.2%

Major Reagent

Rat monoclonal antibody against human p53 DO-1, S-P kit were both from Santa Cruz Biotechnology.

Method

Immunohistochemical method (S-P method): 5 μm sections were cut from paraffin-embedded tissues, and routinely dewaxed in dimethlybenzene, hydrated in grade alcohol, p53DO-1 was 1:200 diluted. Sections were stained according to the S-P kit introduction. Lastly, they were restained in hematoxylin, dehydrated, made transparent, and sealed.

Control: Using 0.01 mol/L PBS instead of monoclonal antibody as blank control; Using known positive section as positive control.

Result judgement: Those with nuclear clearly brown are considered positive; Those only with plasm brown or those with neither plasm nor nuclear brown are considered negative. According to the proportion of positive cells, the positive sections are classified as below: "+" <20%, "++" 20%–50%, "+++" >50%.

Statistics Analysis

Using x^2-test on percentage comparison; Using kaplan-Meier method on accumulated postoperative survival rate.

RESULTS

p53 protein positive rates in intestinal cancer, tissue close to cancer and normal intestinal tissue were analysed by x^2-test, $P<0.01$, the difference had remarkable significance. p53 protein was also positive in such lesions as metastases of liver or lymph node, carcinoma in mucosa, cancer thrombus in vessels, and the most of the expression were strong positive.

DISCUSSION

p53 protein is a kind of phosphoprotein with molecular weight of 53000 dalton, and locates in nuclei. In normal cells, wild-type p53 protein has a short half-life about 3 hours and is low in content, which is seldom detected by routine method. When p53 gene having mutated, the protein confirmation changes, combining with wild-type p53 protein at the same time, its half-life lengthens, and accumulates in malignant cells, easy to be detected.[1] We detected p53 protein in 72 cases of small bowel cancer, the positive rate reached 75%, similar to p53 gene mutation in colonic cancer. Among them 24 cases were "+++", accounting for 44.4% of positive cases, only 4 out of 19 cases of tissue close to cancer were positive, none of which was "+++". One of seven adenomas was positive, no p53 protein expression was detected in normal intestinal tissue (Table 1). The results suggested that p53 gene deletion and mutation may play an important role in the occurrence and development of small bowel cancer.

Table 1. p53 protein expression in small bowel tissue

Group	Cases	+	++	+++	-	Positive rate (%)
Small bowel cancer	72	16	14	24	18	75.0
Tissue close to cancer	19	3	1	0	15	21.1
Metastases	6	1	1	4	0	100.0
Intestinal adenoma	7	1	0	0	6	14.3
Normal tissue	16	0	0	0	16	0.0

Many researches have confirmed that p53 protein expression is related to tumor differential degree. Nucleus staining is extremely clear-cut in poor-differentiated regions while negative in regions with cornified layer. Expression difference is also obvious between cell groups morphologically normal morphology and those with malignant cells, it is close related to tumor differential degree.[2] Present study also found that although there was no significant difference in p53 protein positive rate among those small bowel cancer with different differential degree, the number of positive cells apparently increased in moderate and poor-differentiated adenocarcinoma. Some were high to more than 95%, while the number

was relatively lower in well-differentiated adenocarcinoma. Differential degree correlated with p53 protein expression intensity, while the location of cancer occurrence was not relevant to neither the p53 protein positive rat and nor expression intensity (Table 2).

p53 gene lies in 17p13, so p53 gene deletion and mutation are always studied through detection of 17p mutation. Kern[3] et al. found that: deletion of both 17p and 18q can be used to estimate prognosis in colonic cancer independently and distant metastases closely correlate with deletion of 17p and 18q. Iion[4] et al. have done restricted fragment length polymorphology analysis to 17p, 18q, 22q in colonic cancer, and their result showed that: loss of heterozygosity of 17p, 18q and 22q was more frequent in developing colonic cancer than in cancer in mucosa, LOH of 17p chromosome had close relation to vessel invasion. By immunohistochemistry it was found that: p53 protein expression both in 7 cases of primary cancer and in corresponding to metastases. Some considered the increasing p53 protein expression a marker of development from well-differentiated cancer in mucosa to invasive cancer.[5]

Small bowel cancer occurs latently and lacks characteristic clinical appearance, in the meantime, easy but effective detection method is absent, so early diagnosis seems difficult. The studies on the factors affecting development and metastasis of intestinal cancer appears more important, and directly relates to prognosis, bearing marked clinical significance. Among 72 cases of intestinal cancer in our study, 19 were pathologically confirmed to have metastases to the lymph node, liver and peripheral tissue and organs, whose positive rate and expression intensity of p53 protein increased remarkably when compared with those without metastasis. Both the metastasis of the liver or lymph node and its primary cancer had p53 protein expression, 4 of which were of intense reactivity (Table 2). We also found that p53 protein were positive in such lesions as cancer thrombus in vessels, and invasive cancer below mucosa or in muscle, moreover most of them show strong staining, which suggested that p53 gene mutation closely related to invasion and metastases of small bowel cancer, and is one of the markers of tumor's stronger invasive ability.

Table 2. p53 protein expression and its relationship to small bowel cancer's location, differential degree and metastasis

Different group	Cases	+	++	+++	-	*P*
Duodenal carcinoma	51	12	10	17	12	
Jejunal carcinoma	9	2	2	3	2	>0.05
Ileal carcinoma	12	2	2	4	4	
Well-differentiated	33	11	7	5	10	
Moderate-differentiated	23	3	5	10	5	<0.05
Poor-differentiated	16	1	2	10	3	
Metastasis (+)	19	2	1	12	4	<0.05
Metastasis (-)	53	13	13	13	14	

Table 3. Expression intensity of p53 protein and postoperation survival rate (%)

Intensity of expression	Cases	Postoperation survival time (month)									
		6	12	18	24	30	36	42	48	54	60
-	11	100.0	90.9	90.9	72.7	72.7	72.7	63.6	63.6	45.5	45.5
+	13	100.0	100.0	84.6	76.9	69.2	53.9	53.9	53.9	38.5	38.5
++	13	100.0	92.3	84.6	84.6	69.2	53.9	53.9	53.9	30.8	30.8
+++	20	90.0	75.0	45.0	30.0	20.0	15.0	10.0	10.0	5.0	5.0

Among 64 cases undergoing radical operation, 57 obtained following-up results. According to p53 protein expression and its intensity, they were divided into four groups of " - " "+" "++" "+++" and relation analysis were done on postoperation survival time of each group. The results showed that the accumulated survival rate of patients with positive staining was much lower than that of those negative in p53 protein staining, and accumulated survival rate gradually decreased as p53 protein expression intensity increased (Table 3). p53 gene mutation and deletion affect prognosis significantly. Those with positive especially strong positive p53 protein expression should have treatment after operation, and have long-term following-up.

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