Chinese Journal of Cancer Research 8(4): 263 — 266, 1996

RELATIONSHIP BETWEEN THE MUTATION OF P53 GENE AND INFILTRATION, METASTASIS AND PROGNOSIS OF GASTRIC CARCINOMA

Xin Yan 辛彦 Zhao Fengkai 赵凤凯 Wu Dongying 吴东瑛 Wang Yanping 王艳萍
Xu Lei 徐蕾 Zhang Yinchang 张荫昌

Cancer Institute, China Medical University, Shenyang 110001

Kristin Henry

Department of Histopathology, Charring Cross & Westminster Medical School, University of London, UK

132 gastric carcinomas were examined immunohistochemically with McAb to p53 protein in order to investigate the relationship between the expression of p53 protein and histological differentiation of gastric carcinoma, and to approach the mechanism of infiltration and metastasis of gastric carcinoma. The results showed that nuclear expression of p53 protein was significantly related to tumor size, depth of invasion, lymph node and liver metastases; but not related to histological differentiation. It is suggested that the accumulation of p53 protein was increased with the progression of gastric carcinoma, and therefore the cancer clone with p53 gene mutation may play an important role in the development of tumor invasion and metastasis.

Key words: p53 gene, Tumor suppresser gene. Metastasis, Gastric carcinoma

p53 gene is a tumor suppresser gene which is located on the short arm of chromosome 17p13. Inactivation of the wild p53 gene is considered to be a key event to the carcinogenesis of many malignancies and mutations in the gene are the most common documented genetic alterations in human cancers. In cells, wild type p53 protein has a short half-life (5-20 min) and thus, does not accumulate to detectable levels under normal conditions. Missense mutation in the p53 gene often result in the production of a protein with altered conformation and a prolonged half-life. Simultaneous immuno-histochemical and molecular studies in a limited number of human lung and breast carcinomas have showed that mutations in the p53 gene correspond to tumors immunohistochemically positive for p53 and no mutations being detected in negative tumors.[1-3] As a result, immunohistochemical methods have been successfully used to demonstrate the accumulation of mutant p53 in several types of malignancies. The findings suggested that the assay for mutant p53 has the potential for becoming a useful diagnostic or prognostic marker of malignancy.

The presence of p53 gene mutations and overexpression of p53 protein in gastric carcinomas has been reported,[4,5] but there has been no report on the study of relationship between the expression of p53 protein and the mucinproducing functional differentiation or liver/ovary/adrenal metastases of

Accepted August 30, 1996
This project was supported by the National Natural Sciences Foundation of China.

gastric carcinomas. In the present study, 132 gastric carcinomas were examined immunohistochemically with the monoclonal antibody (DAKO M7001) for the overexpression of p53 protein, and the results were compared with the histological differentiation, growth pattern, depth of invasion, size of tumor, mucin-producing functional differentiation of gastric carcinomas. And so did with the lymph node, liver, ovary and adrenal metastases of gastric carcinomas.

MATERIALS AND METHODS

Seventy-five frozen and 17 formalin-fixed paraffin-embedded tissue (FFPET) blocks of gastric carcinomas came from the Cancer Institute of China Medical University dating 1987-1991; other 40 FFPET materials from the Department of Histopathology of Charring Cross Hospital dating 1987-1991. Among these 132 cases, 100 with lymph node, 12 with liver, 1 with adrenal and 4 with ovary metastases. The blocks from primary and metastatic tumors were chosen from each case, 5 μm cryostat sections from frozen material and routine sections from FFPET material were cut for histological analysis, immunostain of p53 protein and mucin-histochemistry of AB/PAS.

McAb to human p53 protein (DAKO-p53, DO-7, M7001) was used in this study to identify the overexpression of p53 protein. Sections were immunostained using the avidin-biotin-peroxidase complex method, and the pressure cooking was used to unmask p53 antigen in FFPET materials.[6] The mucin-histochemistry was performed by the routine AB/PAS technique.

Evaluation of immunostain. Four semi-quantitative classes were used to describe the number of immunostained cells: Negative (−): none; Weakly positive(+): less than 10% of the cells; Moderately positive (++): 10 — 50% of the cells; Strongly positive (+++): more than 50% of the cells.

RESULTS

The p53 protein was identified in 43 out of 75 frozen materials (57.3%) and in 37 out of 57 FFPET materials (64.9%) of primary gastric carcinomas. There was no significant difference between the expressions of p53 protein in frozen and FFPET sections. Immunostained analysis showed that the expression of p53 protein was significantly related to the size of primary tumor, depth of invasion, lymph node and liver metastases (Tables 1-3, Figure 1,2). In most of primary tumors of gastric carcinomas with liver metastases, there were some small veins which were invaded by carcinoma cells, furthermore, these cancer cells showed strong positivity to p53 protein (Figure 3). 1 gastric carcinoma with adrenal metastasis also showed strong positivity to p53 protein in both primary and metastatic tumors.

In addition, only 3 out of 132 non-tumor stomach mucosa adjacent to the tumors showed positivity to p53 protein, 2 out of which were moderate dysplasia of gastric mucosa and 1 was mild hyperplysia of sqaumous epithelium of the esophagus.

Table 1. Relationship between the expression f p53 protein and the size of primary tumors of gastric carcinomas (n=75)

Diameter of tumor	n	Nuclear expression of p53 protein	
		+ ~ +++ (%)	− (%)
< 4 cm	20	6 (30.0)	14 (70.0)
≥ 4 cm	55	37 (67.3)*	18 (32.7)

* $P<0.01$

Table 2. Relationship between the expression of p53 protein and the depth of invasion of gastric carcinoma tissues (n=132)

Depth of invasion	n	Nuclear expression of p53 protein	
		+ ~ +++ (%)	− (%)
Subserosa	76	38 (50.0)	38 (50.0)
Intoserosa	56	42 (75.0)*	14 (25.0)

* $P<0.05$

Table 3. Relationship between the expression of p53 protein and the lymph node metastases of gastric carcinomas (n=132)

Lymph node metastases	n	Nuclear expression of p53 protein	
		+ ~ +++ (%)	− (%)
Positive	100	68 (68.0)*	32 (32.0)
Negative	32	12 (37.5)	20 (62.5)

* $P<0.0$

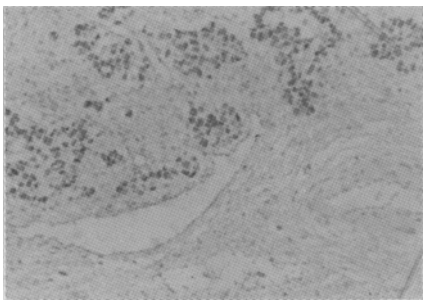

Fig 1. Stomach cancer with liver metastasis. Almost all of the cancer cells of the primary tumor are p53 protein positive, some of which invaded the wall of a small vein (Center). FFPET section 200x.

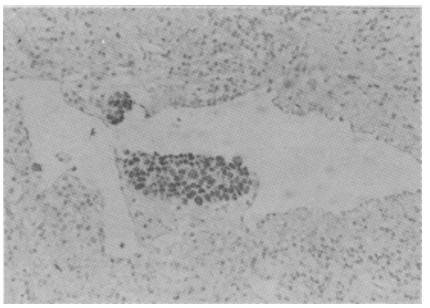

Fig 2. Stomach cancer with liver metastasis (the same case as Fig 1). The cancer cells of the cancerous emboli sticking to the vein show strongly positive reaction to p53 protein. FFPET section 200x.

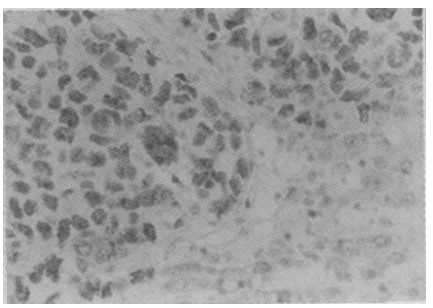

Fig 3. Stomach cancer with liver metastasis (The same case as Fig 1). The cancer cells of metastatic cancer cells in the liver are p53 protein positive (left), but the liver cells are negative (right). FFPET section 400x.

DISCUSSION

General molecular biology techniques such as polymerase chain reaction[7] and DNA sequencing are not suitable for routine clinical applications because they are expensive, difficult to perform, require specialized equipment and also time consuming. The ELISA assay provides an alternative method of detecting and measuring the levels of p53 protein in tumor samples, which is simple and can be easily carried out. But unfortunately ELISA.[8] p53 immunohistochemistry is sensitive, fast and simple and can provide strong evidence of mutations of p53 gene. In the case of p53, immunostain permits not only the identification of tumor with aberrant accumulation of the protein, but also the subclassification of the p53-positive lesions according to their characteristic staining patterns and the proportion of positive tumor cells within each lesion. But there are some reports which showed that some physical factors such as prolonged fixation time and heating of the sections to aid their adherence to the slides deleteriously reduced the intensity of the p53 reaction.[9] The results of our study showed that pressure cooking is very helpful to unmask the p53 antigen in FFPET sections and can increase the intensity of p53 immunoreaction. In our study, p53 protein was identified in 43 cases of 75 frozen materials, while in 37 out of 57 FFPET materials which showed no p53 immunoreaction without using the pressure cooking. The pressure cooking here provided a much more simple quicker and cheaper tool than microwave treatment to unmask the p53 antigen. Patients with gastric carcinoma generally have a very poor 5-year survival time and although 'curative' surgical resection of the tumor and involved tissue with extensive lympha-denectomy significantly improves prognosis,[10,11] recurrence and postoperative mortality are still high.[12,13] However, there are a small number of patients in whom long-term survival can be achieved. [14,15] Pathological staging, as assessed by the depth of invasion, lymph node involvement and distant organ metastases through the blood vascular system, is currently considered the most valuable method of predicting prognosis in patients with gastric carcinoma. The results of our study indicated that the expression of p53 protein was closely related to the size of primary tumor, depth of invasion in gastric wall, lymph node involvement and liver metastasis. In addition, a case with adrenal metastasis also showed

strong positive reaction to p53 protein. A hypothesis for cancer development is that mutations in p53 gene may provide the cell with a proliferative advantage, this would imply that the cancer clone with p53 gene mutations should expand and the number of cells with detectable protein levels should increase with tumor progression. The findings in this study showed agreement with this hypothesis. Authors, therefore, considered that the expression of p53 protein could be used clinically as a prognostic marker to detect the patients with the gastric carcinoma at late stage and with poor survival.

Dysplasia of gastric mucosa has been considered as a precancerous lesion of gastric carcinoma.[16] In this study, 2 cases of moderate dysplasia of gastric mucosa showed positive reaction to p53 protein, which suggested that the mutation of p53 gene might have developed in the precancerous lesion of gastric mucosa and might play an important role in the canceration of gastric mucosa.

In addition, 1 case with mild hyperplasia of sqaumous epithelium of the oesophagus adjacent to the gastric carcinoma located at the cardia area showed scattered positive cells of p53 protein. The significance of scattered staining in simple hyperplasia is uncertain. This may merely represent sporadic mutations or increased accumulation of wild-type p53 in isolated cells and may be related to the DNA repair mechanism.[17] Further study in this field is needed.

REFERENCES

1. Iggo R, et al. Increased expression of mutant forms of p53 oncogen nin primary lung cancer. Lancet 1990; 335: 675.
2. Verly JM, et al. Loss of chromosomes 17p13 sequences and mutation of p53 in human carcinomas. Oncogene 1991; 6: 413.
3. Batek J, et al. Genetic and immunohistochemical analysis of mutant p53 in human breast cancer cell lines. Oncogene 1990; 5: 893.
4. Martin HM, et al. p53 expression and prognosis in gastric carcinoma. Int J Cancer 1992; 50: 859.
5. Seruca R, et al. p53 mutations in gastric carcinomas. Br J Cancer 1992; 65: 708.
6. Norton AJ, et al. Brief high-temperature heat denaturation (Presssure Cooking): A simple and effective method of antigen retrival for routinely tissues. J Pathol 1994; 173: 371.
7. Sidranskey D, et al. Identifications of p53 mutations in bladder cancer and urine samples. science 1991; 152: 706.
8. Joypaul BV, et al. Enzyme-linked immunosorbent assay for p53 in gastrointestinal malignancy: comparison with immunohistochemistry. Histopathol 1993; 23: 465.
9. Midgley CA, et al. Analysis of p53 expression in human tumors: an antibody ransed against human p53 expressed in E.coli. Cell Sci 1992; 101: 183.
10. Craven JL, et al. Radical surgery for gastric cancer. In: Preece PE, et al. eds. Cancer of the stomach. London: Grune and stratton. 1986; 165-188.
11. Adeshek K, et al. Cancer of the stomach. Review of consecutive ten-year intervals. Ann Surg 1979; 186: 6.
12. Meyer HJ, et al. Patterns of recurrence in relation to the rapeutic strategy in gastric cancer. Scand J Gastroenterol 1987; 22 (Suppl 133): 45.
13. Rohde H, et al. What causes in-hospital mortality of surgical patients with cancer of the stomach? Scand J Gastroenterol 1987; 22 (Suppl 133): 76.
14. Remine WH, et al. Long-term survival (10 to 56 years) after surgery for carcinoma of the stomach. Am J Surg 1969; 117: 177.
15. Korenaga D, et al. Long-term survival in Japanese patients with far advanced carcinoma of the stomach. World J Surg 1988; 12: 236.
16. Zhang YC, et al. Precancerous conditions and lesions of the stomach. Springer-Verlag, Heideberg 1993; 1.
17. Hall PA, et al. High levels of p53 protein in UV-irradiated normal human skin. Oncogen 1993; 8L 203.