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Significance and expression of Bax, Survivin and p53 in gastric carcinoma and precancerous lesions using tissue microarray*

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Abstract ***Objective:*** To explore the relationship between expressions of apoptosis-related protein Bax, Survivin and p53 and the molecular mechanisms of carcinogenesis and progression of gastric carcinoma. ***Methods:*** Tissue microarray and immunohistochemistry were used in this study. ***Results:*** The positive rate of Bax protein in gastric cancer (17.7%,17/96) was significantly lower than those in adjacent normal mucosa (51%), intestinal metaplasia (69.2%) and dysplasia (75%), $P < 0.01$. The positive rate of Survivin expression in gastric cancer (80.6%, 89/98) was significantly higher than that in adjacent normal mucosa (3.9%), $P < 0.01$. The positive rates of Survivin expression in tumors with different organ metastases (in lymph node metastasis 86.2%, liver 100% and ovarian 100%) were statistically higher than in tumors without metastasis (64.3%), $P < 0.05$. Bax expression was correlated with Survivin but not with mp53 that was closely related to Survivin expression ($P < 0.05$) in gastric cancer. ***Conclusion:*** The abnormal expressions of Bax, Survivin and mp53 were correlated with the tumorigenesis and progression of gastric carcinoma. P53 and Survivin genes may share the similar mechanism in regulating cell apoptosis, and because of the mutation, p53 gene may lower its down-regulation to Survivin expression.

Key words Bax; Survivin; p53; gastric neoplasm; tissue microarray

On the process of the malignant tumor，the mechanism of the cell apoptosis is related to all the aspects of the apoptosis signal pathway, including the abnormal of the apoptosis inhibiting gene and the apoptosis activating gene. Gastric carcinoma is one of the commonest malignancies in China, and even in the world. However, the molecular mechanism of carcinogenesis of gastric carcinoma remained elusive. In this study, we used the tissue microarray and immunohistochemistry to detect the protein expressions of apoptosis inhibiting gene Bax and apoptosis regulating gene p53 in the gastric carcinoma tissue and the precancerous lesion tissue, and explore the relationship among the protein expressions of the apoptosis inhibiting gene Survivin and p53, Bax. Thus we explored the contribution and the molecular mechanisms of the abnormal apoptosis regulation in the carcinogenesis and progression of gastric carcinoma.

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Materials and methods

Clinical materials

Ninety-eight cases of surgically removed specimens of primary gastric carcinoma were all retrieved from the pathological files of Cancer Institute, China Medical University and Tumor Hospital of Liaoning Province. Tumor stage: 1 case was in early stage, and 97 in advanced stage including 5 in middle stage. Metastasis: 28 were the cases with no metastasis; 65 with lymph node metastasis; 4 with liver metastasis (4 of them accompanying with lymph node metastasis); 1 with ovarian metastasis.

Construction of tissue microarray and immunohistochemistry

A tissue array machine, including manual tissue puncher/arrayer, punches/stylets, recipient block holders, depth stop kit, was bought from Steve Leighton Beecher Instruments, USA. Two blocks of tissue microarrays were constructed, one containing 124 and another containing 101 small cylindrical samples, 1.0 mm each in diameter, from 98 cases of gastric carcinoma, the precancerous lesions (including intestinal metaplasia and dysplasia of the stomach mucosa) and matched adjacent normal mucosa. 4 μm thick series sections were then cut and mounted

Table 1 Expressions of Bax and Survivin proteins in gastric carcinoma and precancerous lesions

Group	Bax expression				Survivin expression			
	n	–	+ – +++	*P*	*n*	–	+ – +++	*P*
Normal	49	24	25 (51.0%)	< 0.01	51	49	2 (3.9%)	< 0.01
GC	96	79	17 (17.7%)		98	19	79 (80.6%)	
IM	26	8	18 (69.2%)	< 0.01	35	3	32 (91.4%)[a]	–
Dysplasia	4	1	3 (75.0%)		4	0	4 (100.0%)	

[a] $P < 0.01$ (Exact $\chi^2 = 62.8772$, vs. normal mucosa); GC: gastric carcinoma; IM: intestinal metaplasia

Table 2 The relationship between the expressions of Bax and Survivin, mp53 proteins in gastric carcinoma tissues

	Bax expression			χ^2	*P*
	n	–	+ – +++		
Survivin expression					
–	17	10	7 (85.9%)	7.6386	< 0.01
+ – +++	78	68	10 (41.2%)		
mp53 expression					
–	29	21	8 (27.6%)	8.2070	> 0.05
+ – +++	56	48	8 (10.7%)		

Table 3 The relationship between expressions of Survivin and mp53 proteins in gastric carcinoma tissues

mp53 expression	Survivin expression				Sum	χ^2	*P*
	–	+	++	+++			
–	14	2	8	5	29	24.498	< 0.01
+	0	7	7	6	20		
++	2	3	6	4	15		
+++	3	4	11	5	23		

in 40 ℃ (the carrier plate was treated with 0.1% polylysine) after broiling in 56 ℃ for 3 h, the sections could be moved to 37 ℃ attemperator overnight, stored at room temperature for use.

The Envision immunohistochemical staining method was used in this study. The rabbit against human Survivin and Bax polyclonal antibodies were bought from Maixin Company (Fuzhou, China). Monoclonal antibody against p53 was from Boster Biotech Company (Wuhan, China), and working dilution was 1:100. DAB coloration, hematoxylin counterstained, 20 mm × 40 mm cover glass mounting. Known positive tissue sections were used for positive controls; for negative controls, sections were incubated with 0.01 M PBS instead of the primary antibodies.

Judging the results of immunohistochemistry staining

Brown granules clearly existing in cytoplasm or nucleus were considered as positive for Survivin, and Bax proteins, in nucleus for mp53 protein expression. Positive rate was assessed by the percent of positive cells in all counted cells (in 2 randomly chosen fields, 400 ×) and the grades were: negative (–), positive rate ≤ 5%; weakly positive (+): 6%–25%; moderately positive (++): 26%–50%; strongly positive (+++): > 50%.

Statistical analysis

All data were analyzed by SPSS 12.0 statistical software, and a value of $P < 0.05$ was considered statistically significant.

Results

The positive rate of apoptosis-inducing protein Bax expression was 17.7% (17/96), significantly lower than that in adjacent normal mucosa, intestinal metaplasia and dysplasia (51%, 69.2%, 75%, respectively), $P < 0.01$. The positive rates of Survivin expression in tumors with metastases (in lymph node metastasis 86.2%, liver metastasis 100% and ovarian metastasis 100%) were significantly higher than that in tumors without metastasis (64.3%), $P < 0.01$. The Bax expression in gastric carcinoma was negatively correlated with Survivin expression in gastric cancer tissues ($P < 0.05$), while, was not related to mp53 protein expression ($P > 0.05$). Expression of Survivin protein was positively related to mp53 protein expression ($P < 0.05$; Table 1–3 and Fig. 1).

Discussion

Bax gene is an important member of the Bcl-2 family inducing cell apoptosis. As a transcription target of the p53, Bax was induced by p53 [1–4], then transferred from enchylema to mitochondrial outer membrane to induce the MMP expression. Bax may be an important intermediary agent in the cell apoptosis signal pathway depended on p53. Transcribed Bax could modify the malignant phenotype of the tumor cell, and the mutant Bax was generally detected in many malignancies, suggesting that Bax is a tumor-suppressive gene. Our study found that the positive rate of Bax expression in normal gastric mucosa, was higher than that in intestinal metaplasia (69.2%) and dysplasia (75%), but Bax protein in gastric cancer expressed significantly lower (17.7%), which suggested that apoptosis inducing protein Bax was of great importance to maintain the normal histological structure and function of gastric mucosa. When the gastric mucosa becomes intestinal metaplasia and dysplasia by adverse factors, Bax protein expression increases and induces cell apoptosis to

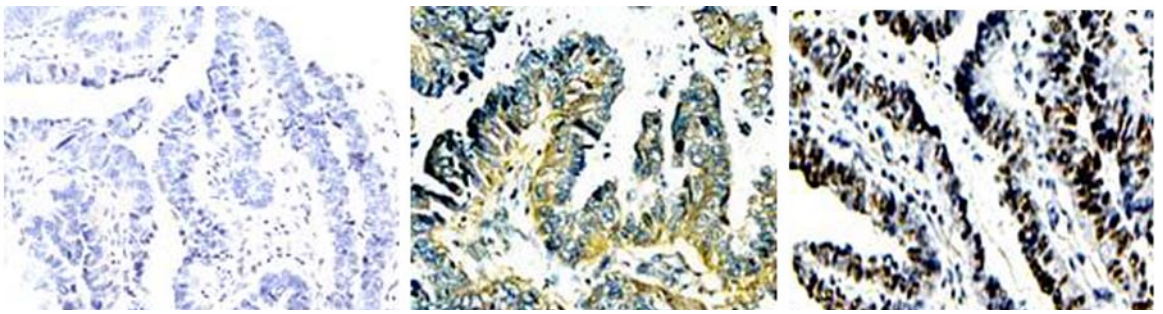

Fig. 1 Bax protein could not be detected (left), Survivin moderately expressed (middle) and mp53 protein strongly expressed (right) in the same case of gastric papillary adenocarcinoma. Envision × 400, × 200 (right)

recur the mucosa. When the damage can not be recurred and proceeds to cancerization, Bax protein expression significantly decreases, and lower expression of Bax was closely correlated with the lymph node metastasis in gastric cancer, which showed that the abnormal expression of apoptosis inducing protein Bax was correlated with the cancerization of the gastric mucosa and the progression of gastric carcinoma. It would be a new molecular biomarker in predicting the tumorigenesis and metastasis of gastric carcinoma. In our research, the positive rate of Bax expression in the group of Survivin positive expression was 41.2%, significantly lower than that in the group of Survivin negative expression (85.9%, $P < 0.01$), but Bax expression did not correlate to mp53 expression ($P > 0.05$), which showed that the low expression of Bax gene in tumorigenesis and the progression of gastric carcinoma may be regulated by other apoptosis correlated genes.

The tumor-suppressive gene p53 plays a key role in regulating cell apoptosis. When the cell damage was too serious to be recurred, wild-type p53 as a apoptosis activating gene could active with p53 negative response element (PNRE) in the 5'-nontranslated domain of Bcl-2 to inhibit Bcl-2 expression and promote apoptosis, thus prevented malignant cell growth and survival [5, 6]. P53 gene was mutated and deleted frequently (about 50%) in human malignant tumors, which indicated that it played an important role in tumorigenesis. P53 gene was mutated frequently in gastric carcinoma, the abnormal expression of p53 was correlated with the tumorigenesis and progression of gastric carcinoma. In our study, there was a closely correlation between Survivin and mp53 expressions in gastric cancer tissues, the mechanism may be as follows: Survivin gene is located in chromatosome 17q25, which is not stable, this site may be involved in t(14;18) shift and induces Bcl-2 gene activation. Bcl-2 gene can prevent cytochrome C releasing from chondriosome to cytoplasm and regulate cell apoptosis. Survivin and Bcl-2 genes were regulated through the similar promoter sequence, therefore, we could presume that p53 and Survivin genes may share the similar mechanism to regulate transcription [7, 8]. P53 gene was mutated frequently in most malignant tumors, meanwhile, abnormal high expression of Survivin gene was also detected in most cancers. It was concluded that because of the mutation, p53 gene may fail its down-regulation to Survivin expression.

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