Relationship between apoptosis and p53 protein expression in squamous cell carcinoma of the esophagus

ORIGINAL ARTICLE Annals of Cancer Research and Therapy

Masatoshi Hasegawa · Michitaka Yamakawa · Norio Mitsuhashi · Tatsuya Ohno
Reiko Imai · Rie Sato · Kazumi Shiojima · Hideo Niibe*  

The relationship between apoptosis and p53 protein expression in squamous cell carcinoma of the esophagus was investigated immunohistochemically in 47 biopsy specimens from 24 patients. Sixteen of the specimens were well differentiated, 11 were moderately differentiated, and 20 were poorly differentiated. The TUNEL method was used for detecting apoptosis. TUNEL-positive condensed nuclei showing characteristic morphological features were considered to indicate apoptosis. p53 protein expression was studied immunohistochemically. One thousand tumor cells were evaluated in each specimen. The incidence of apoptosis was less than 1% except in one specimen. More than 50% of the tumor cells were p53-positive in 33 specimens, while less than 10% of the tumor cells were positive in 14 specimens. There was no significant difference in the incidence of apoptosis or in the mean percentage of p53-positive cells between the 3 histological subtypes. There was also no significant correlation between the incidence of apoptosis and the extent of p53 protein expression.

*p53 expression is known to play an important part in the cell cycle1), and it mediates the induction of apoptosis or recovery from DNA damage2,3). However, when mutant-type p53 protein is expressed, these functions are often blocked. Many scientists have studied the relationship between cancer and p53 protein expression, and a high percentage of squamous cell carcinomas of the esophagus have been found to show p53 protein overexpression4,5). However, p53 expression is not always correlated with the prognosis. In addition, a few reports have recently suggested that there was no significant correlation between apoptosis and p53 expression in esophageal cancer6) and in colon cancer7). In contrast, another report indicated a significant inverse correlation between p53 mutation and apoptosis in patients with esophageal cancer treated by chemotherapy and radiotherapy8). The present study was undertaken to clarify the relationship between apoptosis and p53 expression in squamous cell carcinoma of the esophagus by using the TdT-mediated dUTP-biotin nick end labeling (TUNEL) method9) and immunohistochemistry. Even within the same patient, each biopsy specimen was evaluated separately based on the extent of histological differentiation.

Materials and methods

Sixty-five biopsy specimens were taken from 29 patients with untreated squamous cell carcinoma of the esophagus and were fixed in 10% buffered formalin and embedded in paraffin within 3 days for microscopic examination. However, only 47 specimens from 24 patients could be evaluated histologically, because 18 specimens contained less than 1,000 tumor cells and thus did not have sufficient tumor cells to evaluate apoptosis and p53 protein expression according to our criteria. Each specimen was classified according to the extent of histological differentiation. Sixteen of the 47 specimens were classified as "well differentiated", 11 as "moderately differentiated", and 20 as "poorly differentiated" squamous cell carcinoma.

Sections were stained with hematoxylin and eosin (H&E) for light microscopy. An ApopTag in situ apoptosis detection kit (Oncor, MD, USA) was used for the TUNEL method9). Immunohistochemical studies were performed to detect p53 protein expression by using a mouse anti-p53 monoclonal antibody (DO-7; DAKO, Denmark) as the primary antibody. Deparaffinized sections were pretreated in an autoclave at 120°C for 15 min. A mouse anti-p53 monoclonal antibody (DO-7; DAKO, Denmark) was used as the primary antibody.

For microscopic studies, 1,000 tumor cells per specimen were examined and no specimen which contained less than 1,000 tumor cells was evaluated. TUNEL-positive condensed nuclei found scattered among viable tumor

*Department of Radiology and Radiation Oncology, Gunma University School of Medicine  
Correspondence to: Hideo Niibe, Department of Radiology and Radiation Oncology, Gunma University School of Medicine, 3-39-22 Showamachi, Maebashi, Gunma 371, Japan. TEL 027-220-8383, FAX 027-220-8397

Key words: apoptosis, p53 protein, squamous cell carcinoma, esophageal cancer
cells were counted as evidence of apoptosis. Necrotic areas were excluded from evaluation, and even in the viable areas, TUNEL-positive tumor cells with ballooning of the cytoplasm or condensed nuclei accompanied by inflammation were not counted. Irradiated lymphocytes or intestinal crypt cells from mice and irradiated nude mouse ependymoblastoma cells10,11) were used as positive controls for TUNEL analysis. The percentage of p53-positive cells was determined by counting darkly stained nuclei and no weakly stained nuclei were counted.

The relationship between the percentage of p53-positive cells and the incidence of apoptosis was also evaluated.

Results

Condensed TUNEL-positive tumor cell nuclei were rarely found in the viable tumor areas (Fig.1), although many apoptotic cells in the positive control specimens showed an intense nuclear reaction. The incidence of apoptosis was less than 1%, except in one specimen, and there was no significant difference in incidence between well differentiated (mean ± standard error : 0.31 ± 0.29%), moderately differentiated (0.32 ± 0.21%), and poorly differentiated (0.42 ± 0.67%) squamous cell carcinoma (Fig.2).

p53 protein was often demonstrated in the nuclei by immunohistochemistry. More than 50% of the tumor cells were p53-positive in 33 specimens (70%), while less than
Apoptosis and p53 in esophageal cancer

Immunohistochemical staining of p53 protein by streptavidin-biotin method

p53-positive cells were most frequently found at the periphery of the tumor cell nests in well differentiated carcinoma. (× 250)

Relationship between the percentage of p53-positive cells and the incidence of apoptosis in all specimens

No significant correlation between the percentage and incidence was found (r = 0.12, n = 47).

In this study, there was no significant difference in the incidence of apoptosis between well, moderately, and poorly differentiated carcinoma, but the incidence was no more than 1% in all except one specimen. In our previous studies, the incidence of apoptosis was usually low in untreated tumors compared with that after irradiation.

In the present study, we evaluated the percentage of p53-positive cells. More than 50% of the tumor cells were p53-positive in 70% of the specimens, but less than 10% of the tumor cells were positive in 30% of the specimens. No tumor showed a positivity rate between 10% and 50%, so the distribution of p53 was bipolar (Fig. 3). The methods of p53 evaluation in many previous studies using immunohistochemistry have been more simple than ours and specimens were usually classified into two (− and +), or three (−, +, and ++) groups. Therefore, further study of p53 expression is necessary.

Wild-type p53 often mediates the induction of apoptosis after DNA damage, but loss of normal function may be caused by p53 mutation or other mechanisms. In general, wild-type p53 protein is rarely detected due to its short half-life, but the mutant is easily detected by immunohistochemistry because of its prolonged half-life. The anti-p53 antibody (DO-7) that we used is known to react with both the wild-type and mutant-type p53 protein, but most p53 (DO-7) positivity in untreated tumors is considered to be due to the mutant protein.

We also studied p53-positive rates according to the extent of tumor differentiation, but, we could not find any significant difference in the mean percentage of p53-positive cells between well, moderately, and poorly differentiated carcinoma. However, well differentiated carcinoma usually had p53-positive cells at the periphery of the tumor cell nests, while positive cells were present...
homogenously or were scattered in poorly differentiated carcinoma. These findings are in agreement with those of others.4,5)

In the present study, we evaluated apoptosis, p53 protein expression, and the correlation between them in squamous cell carcinoma of the esophagus. We found no significant correlation between the p53-positive rate and the incidence of apoptosis. However, this result does not necessarily indicate that the apoptosis is p53 independent, because spontaneous apoptosis is not so frequent in the absence of significant DNA damage like that due to irradiation.6,10,11,17,18 In untreated tumors, an inverse correlation between p53 protein expression and apoptosis may exist, though a positive correlation has been reported after DNA damage.14,15,20

In conclusion, we assessed p53 expression and apoptosis histologically in squamous cell carcinoma of the esophagus, but found no significant correlation between them.

This work was supported in part by Grants-in-aid for Cancer Research and Scientific Research from the Ministry of Education, Science and Culture of Japan.

References