Expression of ubiquitin-protein ligases and their substrates in malignant gastric and colonic tumors

Ubiquitin-proteasome pathway (UPP) plays a critical role in protein degradation of eukaryotic cells.\textsuperscript{1}\textsuperscript{,}2\textsuperscript{,}3 Cell-cycle-regulatory proteins such as cyclins, cyclin-dependent kinase inhibitors, and tumor suppressors (e.g., p27, p53) are all substrates of UPP. Therefore, this pathway is involved in tumor generation, development and metastasis. Inhibition of the UPP results in cell death through sensitization of apoptosis. Many pre-clinical studies have demonstrated that proteasome inhibitors such as PS-341\textsuperscript{4}\textsuperscript{,}5\textsuperscript{,}6 may be useful to treat neoplasms. The substrate specificity of UPP is determined by E\textsubscript{3}s (ubiquitin-protein ligases) through recognizing degradation motifs on target proteins.\textsuperscript{1} Different organs may have different E\textsubscript{3}s dominance. We investigated the protein expression of two major ubiquitin-protein ligases (Mdm2, Skp2) and their substrates (p53, p27) in malignant gastrointestinal tumors and their association with pathological grades and lymph node metastasis.

This study was approved by the Ethics Committee of Health Science Center, Beijing Medical University; informed consent was obtained before sample collection. No systemic metastasis was found in these patients. Tumor samples were collected from 30 patients (gastric cancer 15, colon cancer 15) who were diagnosed at surgery to have locally advanced cancer. Samples were collected before chemotherapy was started. Twenty mucosal were obtained from patients undergoing endoscopy for other indications and who had normal findings.

Immunohistochemical study was done on formalin-fixed, paraffin-embedded tissue sections. Each section (4-µm thick) was cut and mounted on aminopropyl triethoxysilane-treated slides. Endogenous peroxidase and nonspecific background staining were blocked by incubating slides with methanol containing 0.3% H\textsubscript{2}O\textsubscript{2}. Slides were washed and then incubated with anti-Skp2 rice monoclonal antibody. Sections were rinsed and then incubated. After being washed, the final products were visualized and sections were counterstained with Mayer’s hematoxylin for 20 s before mounting. Both positive and negative controls were used for each section; gastric carcinoma tissue was used as positive control. Normal rice serum IgG was used as negative control.

Five zones was selected on each slide. 150 tumor cells were counted in each zone. According to the positive nuclear staining percentage of tumor cells, the staining was classified into four groups: negative, +, ++ and +++ when less than 10%, 10%-30%, 31%-60% and >60% of tumor cells showed positive staining. All cases were scored by one person without knowledge of patient status.

The χ\textsuperscript{2} test for significance, Fisher’s exact test and Spearman grade relation analysis were used to test the association between expression levels of Mdm2, Skp2, p53 and p27 and to investigate their expression status according to clinico-pathological characteristics.

Mdm2, Skp2, p53, p27 were mainly expressed in the nucleus in tumor tissue. In normal tissue, p27 was mainly expressed in the nucleus of glandular cells. In tumor tissue, the expression rates of Mdm2, Skp2 and p53 were 60.0% (18/30), 56.7% (17/30) and 56.7 % (17/30), respectively, which were higher than those in normal tissue (p<0.05). The corresponding values for the control group were 4 (20%), 4 (20%) and 2 (10%), respectively. The expression rate of p27 in tumor tissue was 76.7% (23/30), which was lower than in the control group (19/20 [95%]; p<0.05). The expression of Mdm2 was in-
versely related with that of p53 \((r=-0.58, p<0.05)\); the same was the case with Skp2 and p27 \((r=-0.36, p<0.05)\).

No relation was found between the expression of Mdm2, Skp2, p53 and p27 and age and sex of patients, and position and size of tumor. The levels of Mdm2 and Skp2 inversely correlated with pathological grade (Table). The higher the pathological grade, the higher the expression of p27. The levels of Mdm2 and Skp2 in tissue from patients with lymph node metastasis were higher than those without node metastasis; but the expression of p27 was lower in patients with node metastasis.

This study suggests that two major ubiquitin-protein ligases (Mdm2, Skp2) and their substrates (p53, p27) are involved in the development and progression of gastrointestinal cancer. Previous studies have demonstrated the overexpression of Mdm2 or Skp2 in several malignant tumors, including hepatocellular carcinoma, colorectal carcinoma, esophageal squamous cell carcinoma, and lymphoma.\(^1,3,4,6\) Both Mdm2 and Skp2 act in tumor genesis by ubiquitination and subsequent degradation of p53 and p27. Increased Skp2 expression may have a causative role in decreasing p27 expression. Skp2 over-expression and reduced p27 expression correlated with pathological grade. Therefore, evaluation of Skp2, Mdm2 and p27 expression could allow identification of patients with poor prognosis.

Mdm2 over-expression is associated with reduced p53. However, there are two exclusive ways of inactivation of p53: mutational inactivation and functional inactivation. Mutational inactivation of wild-type p53 gene leads to mutated, usually immunohistochemically detectable p53 proteins. At the same time, functional inactivation of wild-type p53 may result from degradation of P53 protein through the UPP, related to over-expressed Mdm2 protein. Hence it is difficult to reveal the protein relationship between Mdm2 and p53 with immunochemistry alone.

The results of our study are preliminary. Further study is necessary to clarify the issues.

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References

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Hepatitis B genotypes virus among chronically infected patients in a tertiary-care hospital in Bangladesh

Eight genotypes (A-H) of hepatitis B virus (HBV) have been identified based on ≥8% divergence of its genome.\(^1\) They have distinct geographical distribution and influence the severity of liver disease.\(^1\) There are no data on HBV genotypes from Bangladesh.

Patients with chronic HBV infection (HBsAg positive for at least 6 months) attending the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, between April and July 2005 were studied prospectively. In all patients, serum transami-