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Expression of retinoblastoma and cyclin D1 in gastric carcinoma

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Abnormal regulation of the cell cycle is a feature of many neoplasms. The role of cell cycle regulators in oncogenesis has been investigated in many human tumors. Alteration of the retinoblastoma (pRb) and cyclin D1 disrupt the Rb pathway and occur in many carcinomas. However the expression of the Rb and cyclin D1 in intestinal type gastric carcinoma is unclear. The purpose of this study was to investigate the expression of Rb and cyclinD1 in resected gastric carcinoma, their adjacent nonneoplastic mucosa and normal gastric mucosa, and finally to provide insights into the role of the Rb and cyclin D1 in gastric carcinogenesis.

We investigated Rb and cyclin D1 expression in 43 patients (32 men, 11 women; mean age: 64) with primary gastric adenocarcinoma and compared the results with adjacent nonneoplastic mucosa. Adjacent nonneoplastic mucosa consisted of atrophy, dysplasia, intestinal metaplasia and gastritis. Expression of Rb was detected in 30 (69.7%) of gastric carcinoma, 18 (41.8%) of the adjacent nonneoplastic mucosa. Expression of cyclinD1 protein was detected in 31 (72%) of gastric carcinoma, 24 (55.8%) of adjacent nonneoplastic mucosa. Expression of Rb and cyclinD1 was not detected in normal gastric mucosa. The positive rate of Rb and cyclin D1 expression in gastric carcinoma was significantly higher than that adjacent nonneoplastic mucosa ($p<0.05$).

There were significant trends for increased expression of Rb and cyclinD1 from nonneoplastic mucosa including atrophy, dyplasia, intestinal metaplasia and gastritis to carcinoma. These results suggested that positive expression of pRb and cyclinD1 might be an early event in gastric carcinoma and it tend to begin at precursor lesions and maintain throughout the progression of infiltration.

Key words: Retinoblastoma, cyclin D1, gastric carcinoma, dysplasia, atrophy, intestinal metaplasia

Malignant transformation results from a series of genetic alterations that lead to aberrant regulation of cell division, cell death and maintenance of genomic integrity. Gastric carcinoma is a common malignancy and still remains a major public health issue. At the molecular level gastric tumors arise from multiple genetic alterations that involve oncogenes, tumor supressor genes, cell cycle regulators, adhesion molecules, DNA repair genes. However, its pathogenesis is still unknown. Recent advances in genomic science have enabled us to uncover the detailed molecular mechanism of gastric carcinogenesis and its progression [1–9].

Abnormalities in cell cycle regulators are involved in gastric carcinogenesis. During the cell cycle, the progression from G1 phase to S phase is essential and critical. Cyclin D1 and retinoblastoma protein (pRb) play major role in the progression [10]. Cyclin D1 is a protooncogene, plays a positive role in the cell cycle. The expression of cyclinD1 is an early event and major targets of cyclinD/cyclin dependent kinase (CDK) complexes are the Rb. The Rb encoding the 105 kd nuclear phospoprotein, maps to chromosome13, band 13q14, and is a prototypical tumor suppressor gene. Phosphorylated pRb, stimulated by the cyclin D/CDK complex, releases E2F transcription factors which activate genes involved in DNA synthesis [3, 9]. Disruption of this so called 'pRb pathway' is a critical event in many tumors and resulted from overexpression of cyclinD1 or by inactivation of Rb function [1–7,10]. Although the functional loss of Rb gene and overexpression of cyclin D1 have been implicated in adverse group of human malignancies, the role of the Rb pathway in gastric carcinomas is still unclear [4–6, 8, 11–16].

Gastric adenocarcinoma, according to Lauren's classification, has been classified as intestinal and diffuse type [4, 11]. Intestinal type of adenocarcinoma follows the pathologic sequential steps of atrophic gastritis, intestinal metaplasia (IM),

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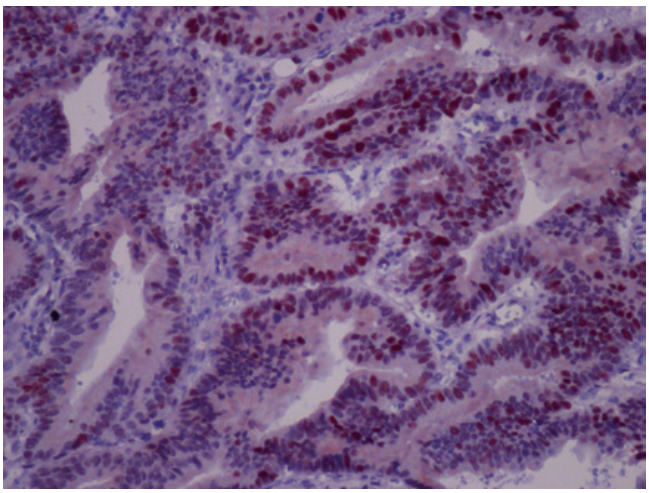

Figure 1. Retinoblastoma positive cells in gastric carcinoma (IHC X100)

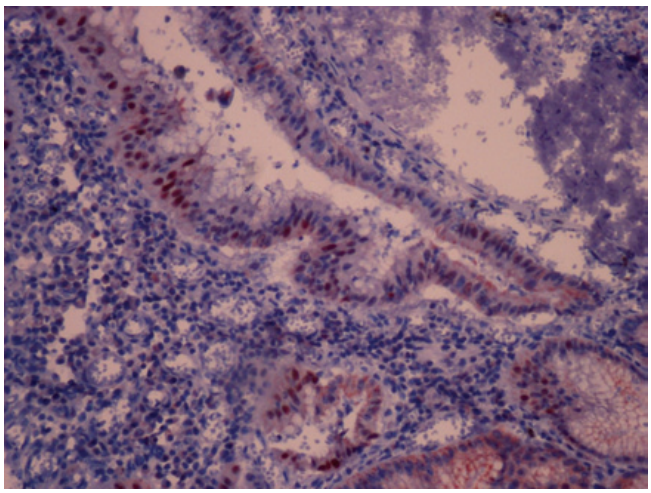

Figure 2. Adjacent to carcinoma dysplastic cells positive with retinoblastoma (IHCX100)

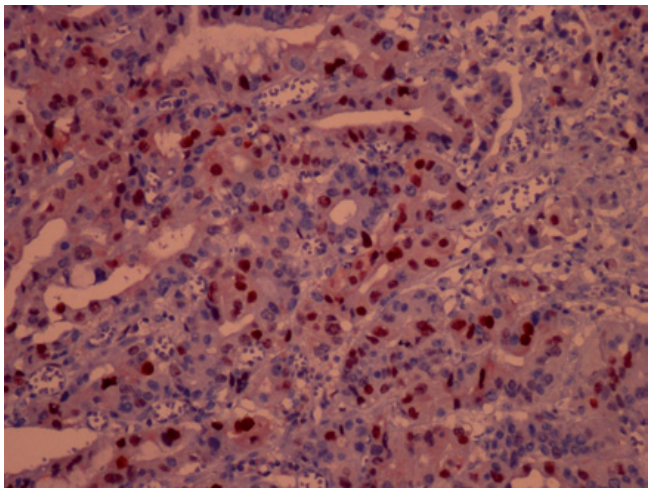

Figure 3. CyclinD1 positive cells in gastric carcinoma (IHCX 100)

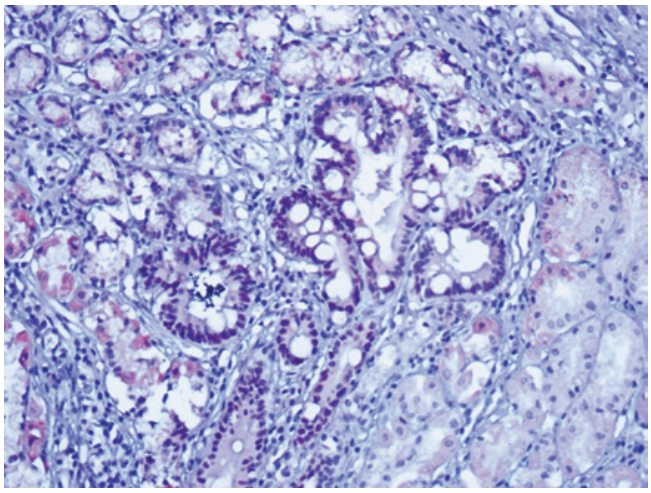

Figure 4. Intestinal metaplasia and positive reaction with Cyclin D1 (IHCX100)

dysplasia and carcinoma. The less common diffuse type correlated with chronic gastritis [1, 4, 5].The current study was designed to examine alterations in pRb and cyclin D1 immunohistochemically in intestinal type of gastric carcinoma and adjacent nonneoplastic mucosa including dysplasia and IM and atrophy. Our aim was to establish whether the immunohistochemical expression of pRb and cyclin D1 have some value for the diagnosis of early stages of gastric carcinoma.

Materials and methods

A total of 43 gastrectomy specimens obtained from gastric carcinoma cases between January 2004 and December 2006 were investigated. Localization of the tumors was antrum or corpus. Hematoxylin and eosin stained slides of intestinal type adenocarcinoma were estimated by histology. Clinicopathologic parameters such as age, sex, histological grade, histological type, serosa invasion, lymph node metastasis, were determined. Nonneoplastic mucosa adjacent to carcinoma including, IM, dysplasia, atrophy and Helicobacter Pylori (H. Pylori) were described. Double staining with periodic acid schiff (PAS) Alcian blue at pH 2,5 was used to establish intestinal metaplasia. Also 20 of the gastric mucosa with normal histology were examined as control group. Immunohistochemical evaluation with Rb and cyclinD1 was performed on normal mucosa, gastric adenocarcinoma and adjacent nonneoplastic mucosa.

Immunohistochemistry. Paraffin sections from each case including neoplastic tissue and adjacent nonneoplastic mucosa were carefully selected. Sections were deparaffinized in xylene and dehydrated through graded concentrations of ethanol. After the blocking of endogenous peroxidase activity with 3% hydrogen peroxide for 15 minute, the sections were heated in 0.01 mol/l citrate buffer in a microwave pressure cooker for 20 minutes. The slides were allowed to cool to room temperature, and non-specific binding was blocked with normal horse serum for 20 minutes at room temperature. The sections were further incubated with the primary antibody against Rb, Clone1F8 (Labvision, Neomarkers) and cyclin D1 CloneSP4 (Lab vision, Neomarkers) for 30 minutes and then primary antibodies against Rb and cyclin D1 were applied.The sections were then stained using the avidin-biotin complex (ABC) immunoperoxidase technique employing commercially available reagent (ABC kit, Labvision, USA), for demonstration of binding sites AEC chromogen was applied. Phosphate buffered saline was used for rinsing between each step and finally all sections were counterstained with Mayer's hematoxylin.

Evaluation of Rb and Cyclin D1. Positive staining was defined as nuclear, cytoplasmic staining was considered nonspecific and ignored. Staining intensity was not recorded. The degree of immunopositivity was evaluated semiquantitaively. The percentage of tumor cell nuclei with positive staining was evaluated as the mean relative nuclear positive staining on at least 10 fields observed at magnification x 40 and 1000 cells was counted and then scored. Scoring of immunostained was categorized as follows: negative, <10%cells stained; positive,>10% cells stained.

Statistical analysis. Chi-square test was used for comparison between groups. All data were calculated with SPSS for windows. $p<0.05$ was considered to be statistically significant.

Results

Thirty two of the 43 patients were men and 11 were women with mean age 64. All of the tumors were intestinal type adenocarcinoma. 6 tumors (13.9%) were grade1, 17 (39.5%) grade2, 20(46.6%) grade3. Serosa invasion and lymph node metastasis were present in 30 (72%) and 36 (83.7) of the cases respectively. Gastritis was detected in all of the cases. Atrophy of mucosaadjacent to carcinoma was observed in 33 (76.7%) of the cases. Dysplasia and IM were present in 31 ((72%) and 34 (79%) cases respectively. 20 of the 43 cases included all three features which are dysplasia, IM and atrophy. Clinicopathologic and histopathologic features of the cases were summarized in Table 1.

Expression of pRb was detected in 30 (69.7%) of gastric carcinoma, 18 (41.8%) of the adjacent nonneoplastic mucosa. Immunohistochemistry scoring results were as follow; Mean relative nuclear positive staining of Rb was around 60%, and 25-30% in carcinoma and adjacent nonneoplastic mucosa respectively. Expression of cyclin D1 protein was found in 31(72%) of gastric carcinoma, 24 (55.8%) of adjacent nonneoplastic mucosa. Mean relative nuclear positive staining of cyclin D1 was around 65% and 40% in carcinoma and adjacent nonneoplastic mucosa respectively. Expression of pRb and was not detected in normal gastric mucosa. Cyclin D1expression was observed only in scattered cells in normal mucosa but no overexpression was found. The positive rate of pRb and cyclin D1 expression in gastric carcinoma was significantly higher than that in adjacent nonneoplastic mucosa and normal gastric mucosa ($p<0.05$). pRb and cyclin D1 expression were not significantly associated with histopathological features such as grade, serosa invasion and lymph node metastasis and H. Pylori infection($p>0.05$).pRb and cyclin D1 results of the adenocarcinoma and nonneoplastic mucosa were summarized in Table 2.

Table 1. Clinicopathologic and Histopathologic Features of The Cases

Age	64 (40-80) years
Sex	32 men (76.7%), 11 women (23.3%)
Histological grade	Grade 1; 6 (13.9%)
	Grade2; 17 (39.5%)
	Grade3; 20 (46.6%)
Histological Type	Intestinal Adenocarcinoma
Serosa invasion positive	30 cases (72%)
Lymph node metastasis	36 cases (83.7%)
Atrophy	33 cases (76.7%)
Intestinal Metaplasia	34 cases (79%)
Dysplasia	31 cases
H. Pylori Infection	30 cases (72%)

Table 2. Changes of Rb and cyclinD1 in adenocarcinoma and nonneoplastic mucosa

Histopathology	Rb IHC positive cases n (%), p<0.05	Cyclin D1 IHC positive cases n(%), p<0.05
Adenocarcinoma	33 (76.7%)	31 (72%)
Nonneoplastic mucosa	18 (41.8%)	24 (55.8%)

Discussion

Gastric carcinoma occurs in patients throughout the world although the incidence varies geographically [4, 11]. Intestinal type gastric carcinoma predominates in developing countries and in lower socioeconomic groups and the incidence of this type is declining. Pathologic sequential steps of atrophic gastritis, intestinal metaplasia dysplasia are important in gastric intestinal type carcinoma. And widely used endoscopic techniques lead to early detection of precancerous gastric lesions and early gastric carcinoma [4, 5, 7, 11].

Inactivating mutations of Rb disrupt the pathway and occur in many caners [1, 3, 8]. The role of the Rb pathway in gastric carcinogenesis is the subject of many papers [1–6, 8, 11–16]. Less information is available on the role of Rb ex-

pression in gastric carcinoma. Some studies showed that Rb protein expression is an important prognostic indicator, in contrast some studies demonstrated that Rb has no prognostic significance [1–6]. Constancia et al reported that alterations affecting the Rb gen are rather infrequent in human gastric adenocarcinomas [3]. Some studies demonstrated that in distal carcinogenesis Rb expression decreased slightly from normal mucosa to carcinoma [1, 5, 16]. In contrast, from gastritis to dysplasia and carcinoma a progressive pRb increase has also been demonstrated [3, 5, 6, 8].The aim of our study was to examine the expressions of Rb in gastric carcinoma and adjacent nonneoplastic mucosa including precancerous lesions. There was negative staining in normal gastric mucosa. However, positive Rb staining was observed in carcinoma and adjacent nonneoplastic mucosa. There was statistically significant difference between the expression of pRb adjacent nonneoplastic mucosa and carcinoma. In 20 cases all of three features; atrophy, IM and dysplasia were observed adjacent to carcinoma. Especially, within these lesions the positive expression of Rb was significantly high, though lower than carcinoma. The significance of the pRb, whether they are causally related to the initiation of tumors or involved in tumor progression, is also investigated in some papers [3, 4, 6, 13, 16]. Some studies have demonstrated that pRb expression is more frequent in gastric carcinoma with superficial invasion [3, 4, 8]. In the current study depth of invasion, lymph node metastasis and grade did not correlate with pRb expression. We thought that changes within pRb occur in the early stages of gastric carcinogenesis and this feature does not change with tumor progression. Thus pRb expression seems related to the initiation of gastric carcinomas.

Cyclins also play a role in the development of neoplasia and any variations in the cyclins' expression result in pathologies of the cell division, including neoplastic proliferation. There have been few reports on the prognostic value of the cyclin expression in gastric carcinoma [7, 11, 12,14]. In normal human tissues, the expression of cyclin D1 was negative by immunohistochemistry. In our study normal gastric mucosa was negative. Some studies showed that cyclin D1 positive expression was significant and associated with well differentiated tumors [10–12, 14]. Different results for cyclin D1values were recently published. Some of the papers have demonstrated that increased cyclin D1 expression associated with poor prognosis. In contrast, other studies showed slightly cyclinD1 increase in adenocarcinoma and did not show increase in precancerous lesions [8, 11, 14, 17, 18]. Gao et al results demonstrated that positive rates of cyclin D1 in early and advanced gastric carcinoma were similar suggesting positive expression of cyclin D1 might be an early event in gastric carcinoma [10]. In our study cyclin D1 was negative in normal gastric mucosa and positive in carcinoma and adjacent noneoplastic mucosa. Over expression rate of cyclin D1 in carcinoma was higher than that of adjacent nonneoplastic mucosa. As detected in pRb, cyclinD1 expression was also high in the dysplastic mucosa and IM. However as pRb, cyclin D1 expression was also not related with the depth of invasion, lymph node metastasis and serosa invasion. It seems that cyclin D1 expression is an early genetic alteration in gastric carcinoma as it was mentioned in some papers [10–12].

The concurrent expression of these genes in gastric carcinoma is not well established. In the current study, we aimed to investigate the expression of pRb and cyclin D1 in gastric carcinoma and adjacent nonneoplastic mucosa in an effort to explain the significance of precancerous lesions in gastric carcinoma. Taken together, our study does support that dysregulation of cell cycle is a prerequisite for gastric carcinogenesis via Rb pathway. Overexpression of cyclinD1 and pRb has some value for the diagnosis of early stages of gastric carcinoma cases, especially associated with atrophy, IM and dysplasia. However more studies are needed to asses the role of these proteins in the pathogenesis of gastric intestinal type carcinoma. We thought that, the role of the cyclin D1 and pRb in the oncogenesis of gastric carcinoma and precancerous lesions is worth investigating.

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