J Korean Med Sci 2001; 16: 731-5
ISSN 1011-8934

Expressions of p53 and p21 in Primary Gastric Lymphomas

The p21 overexpression is thought to be a consequence of the p53 induced activation of the *p21* gene. The immunohistochemical evaluation of p53 and p21 can be a valuable means of assessing the functional status of the *p53* gene product. We examined the overexpression of p21 and p53 proteins in primary gastric lymphomas and the correlation with prognosis. A total of 32 cases of gastric lymphomas was classified into low-grade lymphomas of mucosa-associated lymphoid tissue type (n=16) and high-grade B-cell lymphomas (n=16). In low-grade lymphomas, only one case showed p53 positivity and all cases were p21-negative. In high-grade lymphomas, seven cases were p53+/p21- (44%), one case was p53+/p21+ (6%), and eight cases were p53-/p21- (50%). The p53+/p21- cases had a much lower percentage of patients sustaining a continuous complete remission state (3/7, 43%) compared with other cases (6/7, 86%). From these results, we concluded that p21 expression is rare in primary gastric lymphomas. Therefore, p53-positive lymphomas can be assumed as having p53 mutation. And combined studies of p53 and p21 may be used as a prognostic indicator in primary gastric high-grade lymphomas.

Key Words : *p21; p53; Lymphoma; Stomach; Prognosis*

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Received : 11 June 2001
Accepted : 3 July 2001

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* The research was conducted by the research fund of Dankook University in 2000.

INTRODUCTION

The control of mammalian cell cycle involves a family of proteins that bind to and inhibit the cyclin-dependent kinases (CDK) (1). Recent biochemical and genetic studies suggest that these CDK inhibitors may act as tumor suppressors (2). They bind to the specific CDKs or CDK-cyclin complexes and inhibit kinase activities contributing to the proper control of the G1-S transition, and thereby resulting in an arrest of the cell cycle (2).

Based on the comparative studies of amino acid sequences and biochemical properties, two classes of CDK inhibitors, INK family and CIP/KIP family (3), have been identified (2). The INK family includes p16, p15, and p18 (2). The CIP/KIP family includes p21, p27, and p57, and compared with INK family members, these inhibitors have a less selective inhibitory effect on many CDK-complexes with a main activity during G1 phase (3).

The p21 (WAF, WAF1) is a small protein that affects the function of most known cyclin/CDK complexes, blocking DNA replication and cell cycle progression into S phase (3). p21 plays a dual cell cycle inhibitory function at the G1 checkpoint, by interacting with cyclin/CDK complexes and also with the DNA polymerase δ replication factor proliferating cell nuclear antigen, binding with the targets on separate domains and inhibiting its activity (2, 3). This pathway is important because it allows the cell to repair DNA rather than replicating DNA errors that may lead to a cellular transformation (2). Recent studies suggest that the *WAF1* gene contain p53 binding sites and be transactivated after a treatment with DNA damaging agents and that the p21 product be a potent downstream effector of p53 tumor suppressor gene function (3).

Because p21 protein is mostly expressed in actively proliferating cells, but rarely in latent cells, p21 expression is well correlated with proliferating index. p21 seems to induce a cell cycle arrest and apoptosis in actively proliferating cells and is an important factor regulating the cell cycle in proliferating cells (4).

Overexpression of p21 results in growth inhibition of colon cancer cells, brain tumor cells, and leukemia cells and can induce a growth arrest and apoptosis of human carcinoma cell lines (1). However, the expression of p21 varies in neoplastic and nonneoplastic lesions of different organs and p21 performs different functions through p53-dependent or independent pathways (4).

Previous studies on non-Hodgkin's lymphomas (NHL) showed that the p53 protein expression is a frequent finding in high-grade NHL with a 27% frequency in high-grade NHL of the mucosa-associated lymphoid tissue (MALT) type,

but not in low-grade cases (5). However, there are few data concerning the p21 expression pattern in primary gastric lymphomas. In this study, we analyzed the expression of p21 in primary gastric B-cell lymphomas by immunohistochemical stains using a p21-specific monoclonal antibody.

MATERIALS AND METHODS

Materials

Paraffin-embedded tissue blocks from 32 cases of primary gastric B-cell lymphomas were retrieved from the Department of Pathology, Yonsei University College of Medicine and Department of Diagnostic Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine in Korea. The diagnosis of low-grade lymphoma of the MALT type was made based on the well-established histologic criteria described by Isaacson and Norton (6). Lymphomas predominantly composed of large transformed cells were diagnosed as high-grade B-cell lymphomas. Their clinical data, including modalities of treatment and outcome, were obtained from hospital charts. The clinical stage was determined according to the Luganos international classification (7).

Immunohistochemistry

Lymphoma samples were tested for p53 and p21 protein expressions with a monoclonal antibody DO-7 (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK; dilution 1:100) and with a monoclonal antibody EA10 (Transduction Laboratories, Lexington, NY; dilution 1:100), respectively. The immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded material. Briefly, sections were deparaffinized in xylene, rehydrated, washed in distilled water, immersed in 10 mM citrate buffer with pH 6, and microwaved (twice for 5 min each). The sections were treated with a 3% H_2O_2 to reduce endogenous peroxidase activity, washed in phosphate-buffered saline, and were subsequently subjected to the primary antibody reaction. Detection of the immunoreactive staining was carried out by the labelled streptavidin biotin method using LSAB kit (DAKO, U.S.A.). The sections were subjected to a color reaction with diaminobenzidine and counterstained with Mayer's hematoxylin.

Cells were considered positive only when a distinct nuclear immunostaining could be shown, excluding cells with faint staining. Immunostained slides were first scanned under a low power magnification (×100) to locate the areas with maximal positive cells and then scored by counting the number of positive tumor cells at ×400 or ×1,000 field. A quantitative evaluation of antigen expression was performed and it was considered as positive if more than 10% of tumor cells were immunostained.

Statistical analysis

Fisher's exact test was used to analyze the correlation between patient's prognosis and immunophenotype. $p<0.05$ was considered to be statistically significant.

RESULTS

Clinical results

Thirty-two cases of primary gastric B-cell lymphomas were included in this study. Sixteen were low-grade gastric lym-

Table 1. Clinical characteristics and immunohistochemical results in primary gastric high-grade B-cell lymphomas

Case	Sex/Age (yr)	Stage	Current Status	Treatment	Survival in months	p53/p21
1	F/36	I_1	Lost follow up	Surgery		-/-
2	M/21	II_1	Complete remission	Surgery + CTx	72	+/-
3	M/33	I_1	Complete remission	Surgery + CTx	58	+/-
4	F/74	IV	Lost follow up	Surgery		-/-
5	F/67	II_1	Lymphoma related death	Surgery + CTx	7	+/-
6	F/53	II_1	Alive with disease	Surgery + CTx	42	+/-
7	M/52	II_1	Lymphoma related death	Surgery + CTx + RTx	18	+/-
8	M/61	I_2	Complete remission	Surgery	32	-/-
9	F/58	II_1	Complete remission	Surgery + CTx	16	+/-
10	F/56	IV	Complete remission	Surgery + CTx	67	-/-
11	F/50	II_1	Complete remission	Surgery + CTx	23	-/-
12	F/56	II_1	Alive with disease	Surgery + CTx	42	+/-
13	F/64	II_1	Alive with disease	Surgery + CTx	32	-/-
14	M/40	II_1	Complete remission	Surgery + CTx	33	+/+
15	M/57	II_1	Complete remission	Surgery + CTx	34	-/-
16	M/41	I_1	Complete remission	Surgery	48	-/-

CTx: chemotherapy, RTx: radiotherapy, +: positive, -: negative

phomas of the MALT type and 16 were high-grade B-cell lymphomas.

The median age of the patients with high-grade lymphomas was 51 yr, and that with low-grade MALT lymphomas was 49 yr. In low-grade MALT lymphomas, 9 of 16 patients presented with stage I1 disease, 6 presented with stage II1, and 1 presented with stage II2 disease. In high-grade B-cell lymphomas, 3 of 16 patients presented with stage I1 disease, 1 patient presented with stage I2 disease, 10 patients presented with stage II1 disease, and 2 patients presented with stage IV disease. Mean survival duration was 38.25 and 37.43 months in low-grade and high-grade lymphomas, respectively. All patients with low-grade lymphomas were alive with no evidence of the disease. Response to treatment (defined as achievement of complete remission) was observed in 9 of 14 (64%) high-grade cases. Five of fourteen (36%) patients

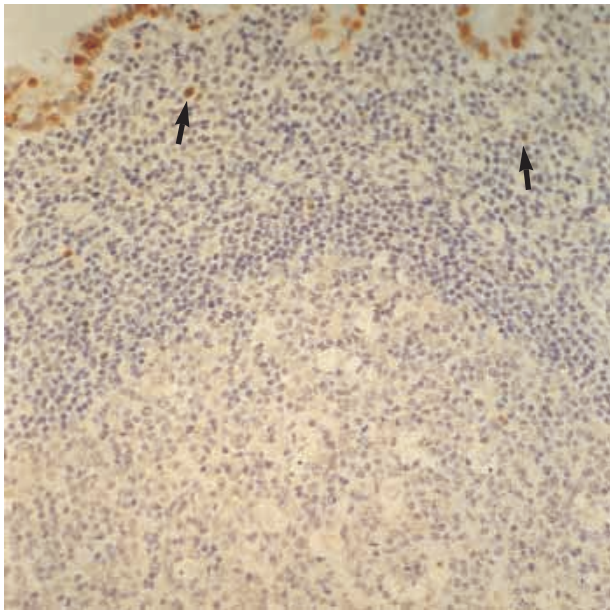

Fig. 1. In Peyer's patch section, rare p21-positive cells are found (arrows) (×200).

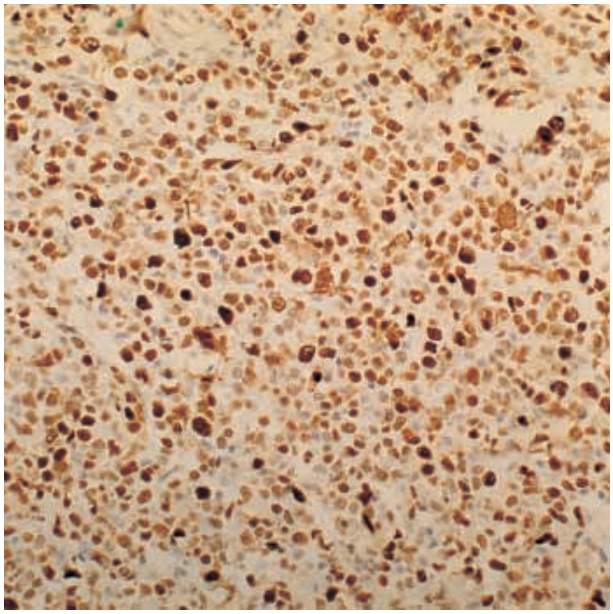

Fig. 3. A case of high-grade gastric B-cell lymphoma shows diffuse and strong immunostaining for p53 (×200).

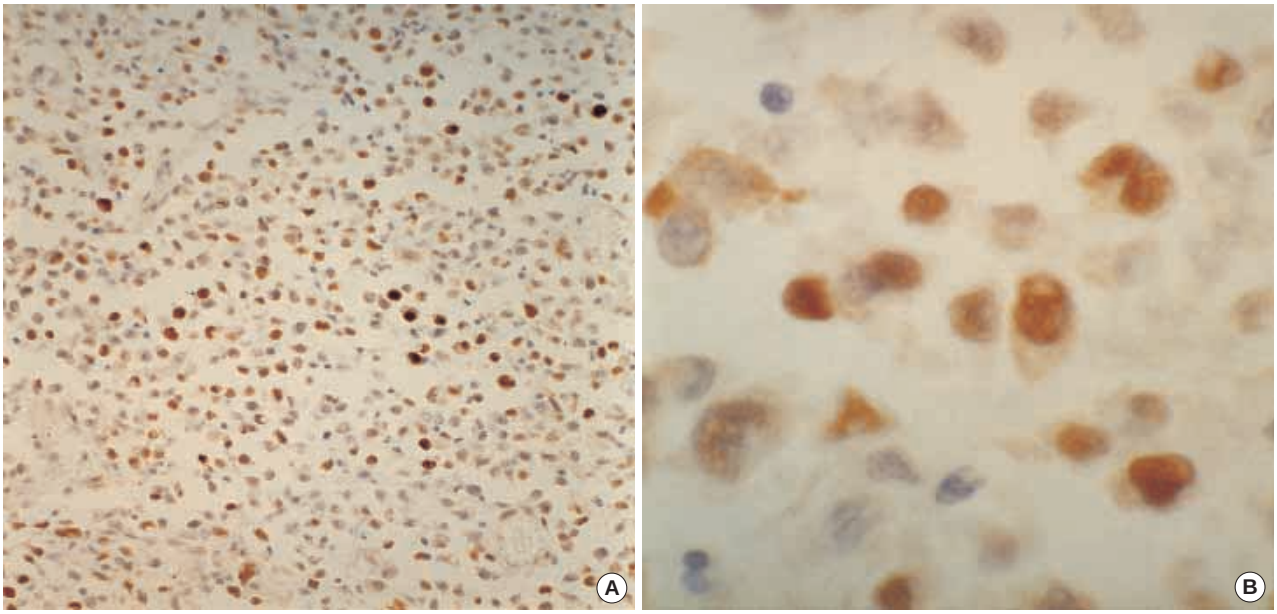

Fig. 2. One case of high-grade gastric B-cell lymphoma shows that more than 10% of tumor cells are positive for p21 protein (A, ×200). These p21-positive tumor cells are clearly demonstrated in an oil immersion view (B, ×1,000).

showed either lymphoma-related death or partial remission of the disease. Clinical characteristics of patients with high-grade lymphomas were summarized in Table 1.

Immunohistochemical analyses

In Peyer's patch as a control, distinct nuclear immunostaining for p21 was observed only in rare cells in the germinal center, mantle and marginal zones. Most epithelial cells were positive and could be used as a positive control (Fig. 1).

In low-grade lymphoma group, only one case showed moderate p21 staining in more than 5% of cells. Another case was only p53-positive. The remaining 14 cases were both p53- and p21-negative. In high-grade lymphoma group, only one case was p21-positive (Fig. 2) and 8 of 16 cases were p53-positive (Fig. 3). The p21-positive case was also positive for p53. In summary, seven cases were p53+/p21-, one case was p53+/p21+, and eight cases were p53-/p21-. Among the five cases of high-grade lymphomas that failed to attain complete remission, four cases were p53+/p21- and one case was p53-/p21- (Table 1).

DISCUSSION

Mutated p53 protein tends to accumulate in the cells and thus can be detected immunohistochemically. It has been a conventional paradigm that p53 accumulation in neoplastic cells is synonymous with p53 mutation. Recently, however, it was demonstrated that a large fraction of NHL cells accumulate a wild type form of p53 at the nuclear level (3). The *WAF1* gene contains p53 binding sites and is transactivated after a treatment with DNA damaging agents and the p21 product is a potent downstream effector of p53 tumor suppressor gene function (1). Mutated p53 does not function as a transcriptional regulator, and thus prevents the induction of p53-dependent p21 synthesis (3). Previous reports revealed that the number of neoplastic cells expressing nuclear p21 was comparable to that of cells with nuclear p53 immunostaining in most p21-positive cases, and a double marker analysis confirmed the colocalization of both p53 and p21 in the same neoplastic cells (1). It is possible to speculate the p21 overexpression observed in neoplastic cells in these cases is a consequence of the physiologic activation of the *WAF1* gene, which is the downstream target of the p53 phosphorylation (1). Overexpression of the wild type p53 protein was associated with the accumulation of p21 with no structural abnormalities (1). Therefore the combined immunohistochemical evaluation of p53 and p21 may be a valuable means of assessing the functional status of the p53 tumor suppressor gene product in NHL (1).

In a reactive lymph node and infant thymus as controls, distinct nuclear staining for p21 was observed only in rare scattered cells (1). It has been reported that the expression of p21 was relatively frequent (18% to 27%) in NHL (1, 3, 5). In case of MALT or gastric lymphoma, all low-grade MALT lymphomas were p21-negative and p53-negative (1, 5). In our study, only 1 out of a total 16 cases of low-grade MALT lymphomas showed p53 positivity, while all 16 cases of low-grade MALT lymphomas were p21-negative, which is consistent with the previous results.

The previous studies revealed that p21 was expressed in 20% to 48% of high-grade gastric or MALT lymphomas (1, 5). In our study, however, only one case showed distinct nuclear staining in more than 10% of tumor cells. We observed that many spindle cells were strongly positive for p21 in many high-grade lymphoma cases. In order to ascertain that the reactive cells were the tumor cells, we performed a double immunostaining for p21 and CD20 in indeterminate cases.

According to a previous study with 25 cases of high-grade B-cell lymphomas, seven cases were p53+/p21- (28%), four cases were p53+/p21+ (16%), one case was p53-/p21+ (4%), and 13 cases were p53-/p21- (52%) (1). Another study with 23 cases of high-grade MALT lymphomas revealed that four cases were p53+/p21- (17%), nine cases were p53+/p21+ (39%), two cases were p53-/p21+ (9%), and eight cases were p53-/ p21- (35%) (5). In the present study, seven cases of high-grade lymphomas were p53+/p21- (44%), one case was p53+/p21+ (6%), and eight cases were p53-/p21- (50%). In comparison with the results in previous studies, the cases showing a p53+/p21- pattern were more frequently found in the present study.

Because the expression pattern of p53+/p21- means the presence of mutated *p53* gene, patients with functional *p53* gene (p53+/p21+, p53-/p21-, and p53-/p21+) and those with a p53+/p21- pattern have been considered to be different in their treatment outcomes (1). However, these findings were neither confirmed nor mentioned in the previous studies concerning NHL, especially primary gastric lymphomas. In the present study, the proportion of patients in the p53+/p21- cases who obtained a continuous complete remission was lower (3/7, 43%) when compared with that of the remaining ones (6/7, 86%), although this difference was not statistically significant (p=0.266).

Although it is well established that the ability of p53 to induce G1 arrest is related to the synthesis of the CDK inhibitor p21, which promotes cell growth arrest, the mechanisms by which p53 induces apoptosis are still largely unknown (1, 3).

p21 has been implicated in the survival response of different cells by p53-independent or p53-dependent manner, rescuing cells from apoptotic cell death (2, 8). In B lymphoma cells, p53-independent elevation of p21 protein may directly interfere with the caspase cascade, thus playing a dual role in both cell cycle progression and apoptosis. Reduction in the induced p21 protein level results in diminished G1 arrest and increased apoptosis in B lymphoma cells (9). In Hodgkin's

lymphoma and small lymphocytic lymphoma, p27 and possibly p21 may be involved in the protection from apoptosis (10).

In malignant lymphoma, it has been suggested that p53 induced p21-mediated growth arrest be somehow overridden in NHL cells, because the cells coexpressing p21 and p53 were the actively proliferating elements (1). Alternatively, it is possible that the expression of p21 may protect the tumor cells from apoptosis in high-grade lymphomas. Studies involving transfection of p21 antisense oligonucleotides in primary gastric high-grade lymphomas are warranted to validate this hypothesis.

In conclusion, p21 expression is rare in primary gastric lymphomas despite the high p53 positivity especially in high-grade cases. Therefore, p53-positive cases can be considered as having p53 mutation in primary gastric high-grade B-cell lymphomas. In addition, the result of the combined immnohistochemical evaluation of p53 and p21 may be used as a prognostic factor in primary gastric high-grade lymphomas, because p53+/p21- cases had lower continuous complete remission rate than other immunophenotypes.

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