The Korean Journal of Pathology 2009; 43: 212-20
DOI: 10.4132/KoreanJPathol.2009.43.3.212

The Expressions of E2F1 and p53 in Gastrointestinal Stromal Tumors and Their Prognostic Significance

Mi Jung Kwon · Eun Sook Nam
Seong Jin Cho · Hye Rim Park[1]
Hyung Sik Shin · Jong Seok Lee[2]
Chan Heun Park[2]
Woon Geon Shin[3]

Departments of Pathology, [2]Surgery, and [3]Internal Medicine, Hallym University of College of Medicine, Kangdong Sacred-Heart Hospital, Seoul; [1]Department of Pathology, Hallym University Sacred-Heart Hospital, Anyang, Korea

Received : October 1, 2008
Accepted : February 6, 2009

Corresponding Author
Eun Sook Nam, M.D.
Department of Pathology, Kangdong Sacred-Heart Hospital, Hallym University Medical College, 445 Gil 1-dong, Gangdong-gu, Seoul 134-701, Korea
Tel: 02-2224-2349
Fax: 02-2224-2214
E-mail: esnam2003@yahoo.co.kr

Background : E2F1 plays a critical role in the G1-to-S phase transition by inducing various genes that encode S phase-activating proteins and that modulate such diverse cellular functions as DNA synthesis, mitosis and apoptosis. The purpose of this study was to assess the E2F1 expression in relation to the clinicopathologic parameters and other tumor markers in gastrointestinal stromal tumors. **Methods :** Immunohistochemical stainings for obtaining the E2F1, p53, and Ki-67 labeling indices were performed on a tissue microarray of 72 gastrointestinal stromal tumor specimens. The clinicopathologic parameters that were analyzed including the risk grade system by Miettinen *et al.* and the disease-free survival (DFS) rate. **Results :** 1) An E2F1 expression was correlated with a larger tumor size, a p53 expression and a shorter period of DFS (p=0.014, p=0.007, and p=0.039). 2) A p53 expression was significantly associated with a high risk grade, a larger tumor size, high mitotic counts and a shorter period of DFS (p=0.003, p=0.044, p<0.001, and p<0.0001). 3) A high-risk grade and the epithelioid type were significantly associated with a shorter period of DFS (p=0.0006 and p=0.0008). **Conclusions :** E2F1, as well as p53, may be a potentially novel independent prognostic factor for predicting a worse outcome for those patients suffering with Gastrointestinal stromal tumors.

Key Words : Gastrointestinal stromal tumor; E2F1 transcription factor; Ki-67 antigen; Tumor suppressor protein p53; Immunohistochemistry

Gastrointestinal stromal tumors (GISTs) comprise 1-2% of all of malignant gastrointestinal tumors.[1] The most common sites are stomach (60%), small intestine (35%), and colorectum (<5%). Approximately 20% to 25% of gastric and 40% to 50% of small intestimal GISTs are clinically malignant.[1] Since GISTs are believed to originate from the interstitial cells of Cajal as suggested by Kindblom *et al.* in 1988,[2] GISTs have been studied for determining their histologic diagnostic criteria, their malignant potential and their prognostic factors. The criteria for malignancy are not very clear if the histologic findings alone are examined because this tumor has a wide spectrum of biologic behavior and the histologic features among different tumor sites are variable.

There is currently no firmly established specific grading or staging system for GISTs. The consensus system proposed by the National Institute of Health in 2001 is the one of the most commonly used grading systems, but the consensus system was not based on specific data sets and they significantly overestimated the biologic potential of gastric GISTs.[3] Therefore, the use of guidelines for gastric and small intestinal GISTs was introduced, as was suggested by Miettinen *et al.* of the Armed Forces Institute of Pathology[3] (the AFIP system). This risk grade system uses the tumor site as a prognostic factor, and this is anticipated to provide more correct prognostic information.

The generally accepted prognostic parameters are the tumor size, the mitotic count, the proliferation index and the tumor site.[4] Several studies have reported other prognostic factors such as the type of c-kit mutation, a high Ki-67 labeling index (LI), the overexpression of cyclin B1, the inactivation of p16, a low expression of p27 and a high expression of p53.[5] The cyclin-dependent kinase inhibitor 2A (CDKN2A) tumor suppressor pathway has recently been reported to be the pathogenesis of various kinds of tumors.[6] A few studies have reported on using cell cycle regulatory proteins in GISTs.[7] E2F1 is a cell cycle regulatory protein of the CDKN2A tumor suppressor pathway, and

it has been recognized as a prognostic factor for various tumors via its genetic and protein levels.[8] There are 8 subtypes in the E2F family, from E2F1 to E2F8.[9,10] They are functionally divided into 2 groups: E2F1, E2F2 and predominantly act as activating transcription factors and E2F3b, E2F4, and E2F5 predominantly act as transcriptional repressors. E2F6, E2F7, and E2F8 have only recently been detected and their functions are not yet clear.[10] In the E2F family, probably only E2F1 seems to be involved in a dual promoting and apoptotic effect.[11] The E2F1 transcription factor plays a key role in G1-to-S phase transition by attracting numerous upstream signals.[12] In normal cellular physiology, E2F1 modulates such diverse cellular functions as DNA synthesis, mitosis and apoptosis.[12] In tumorigenesis, E2F1 acts either as an oncogene or as a tumor suppressor gene depending on the tumor type and the predominant signal.[12] The overexpression of E2F1 is significantly associated with higher tumor proliferation and greater invasive ability. In the previous literature, E2F1 has been shown to acts as an oncogene and it has a tumor-promoting effect in non-small cell lung cancer and breast, thyroid and ovarian epithelial cancers.[12-15] On the other hand, E2F1's tumor suppressor effect has been studied in colon cancer and bladder cancer.[16,17] E2F1 has been shown in some studies to have a tumor-promoting effect in GISTs.[7]

Tissue microarrays (TMA) are a high-throughput method for analyzing large numbers of formalin-fixed, paraffin-embedded materials with minimum cost and effort.[18] We applied TMA technology to immunohistochemically analyze E2F1 and other additional markers in GISTs. In this study, we evaluated the expression of the cell cycle regulatory protein E2F1 and the correlation between the clinicopathologic parameters and the AFIP risk grade system in GISTs.

MATERIALS AND METHODS

Patients, tumor grouping and grading

Among all the patients who were diagnosed with GISTs and who underwent surgical resection for primary GISTs from 1990 to 2007 at Kangdong Sacred-Heart Hospital at the Hallym University College of Medicine, 72 cases that expressed CD117, as assessed by immunohistochemical staining, were enrolled in this study. According to the AFIP risk grade system for gastric and small intestinal GISTs (Table 1), which was based on the long-term follow-up of more than 1,600 patients by Miettinen *et al.,* we classified the patients into the low, intermediate and high-risk groups. The very low and low risk groups were placed together. GISTs arising in the stomach and esophagus were classified by the "gastric grading system" and GISTs arising in small intestine, large intestine, rectum and omentum were classified by the "intestinal grading system". Tumor recurrence and the survival of patients were assessed by outpatient follow-ups and conducting telephone interviews.

Pathological evaluation

All of the surgical specimens were re-evaluated by two authors. The clinical evaluation included the patient's gender, the patient's age, the tumor location and tumor size. Tumor size was evaluated as the greatest dimension taken from the pathology reports. The GISTs were histologically subclassified into 2 subtypes: the spindle and epithelioid subtypes. The amount of mitosis was determined by counting the mitotic activity in 50 adjacent high-power fields (HPF) in the full section of a hematoxylin and eosin (H&E) slide at a magnification of $\times 400$. Tumor necrosis and tumor hemorrhage were determined as being either present or absent.

Creating the tissue microarray block

After reviewing the cases for diagnostic confirmation, the TMA was constructed. The representative area was drawn in a circle. Each paraffin-embedded block that was relevant to an H&E slide was punched out by using a tissue microarray manufacture tool (Quick-Ray^{MT}, Unitma, Seoul, Korea). A 3 mm punch size was used. One core from each paraffin-embedded block was punched out and six cores were embedded in each tissue microarray block in a 3×2 arrangement. To evaluate the order of the cores, a punch of palatine tonsil was embedded ahead of each tissue microarray block.

Immunohistochemical staining

The histologic sections (4 μm) of 12 tissue microarray blocks of the 10% formalin-fixed, paraffin-embedded materials were used for the study. The sections of the TMAs were deparaffinized in xylen and they were rehydrated through a series of graded ethanol solutions. Antigen retrieval was achieved by microwave treatment in 0.01 mol/L citrate buffer (6.0 pH) for two minutes and then the sections were cooled for two hours. Immunohistochemical staining was performed with using the Dako ChemMate Kit according to the manufacturer's instructions. The seven

primary antibodies used were c-kit (polyclonal c-19, 1:200, Santa Cruz biochemistry, CA, USA), CD34 (monoclonal, 1:50, Dako, CA, USA), smooth muscle actin (monoclonal, 1:200, Dako), S-100 protein (polyclonal, 1:400, Dako), Ki-67 (MIB-1, monoclonal, 1:100, Dako), p53 (monoclonal, 1:100, Dako) and E2F1 (monoclonal, 1:200, KH95, Dianova). The sections were incubated with the primary antibodies at room temperature for an hour. Each section was treated sequentially with biotinylated secondary antibody for 20 min and this was followed by streptavidin peroxidase for 20 min.

For the sections of E2F1, we used the DAKO catalyzed signal amplification (CSA) system after the E2F1 primary antibody had been applied for an hour. 3,3′-Diaminobenzidine tetrahydrochloride was used as a chromogen and then a hematoxylin counterstain was applied. A breast cancer section was processed in parallel as a positive control for E2F1.

Analysis and Interpretation of the staining

Interpretation of the staining results was done by two authors. There was close agreement (>90%) between both the investigators. For the cases of disagreement, the final grading was determined by consensus.

Positivity for c-Kit was defined when the staining showed a strong and uniform membrane Golgi-like zone or a cytoplasmic staining pattern that had an intensity equal to an internal control element such as a mast cell.

CD34 positivity was defined as strong and diffuse staining along the cell membrane or in the cytoplasm.

SMA and S-100 positivity was considered as diffuse and intense cytoplasmic staining. The percentage of the tumor cells with positive staining for Ki-67 was scored as the Ki-67 LI. The cut off value of Ki-67 LI was determined as 4.92% when the disease-free survival analysis was evaluated.

For the E2F1 and p53 protein expressions, only nuclear staining was considered positive. Positive cells were counted by monitoring for a relatively uniform staining density. We considered the E2F1 and p53 stainings as positive when >5% of the cancer cell nuclei showed positive immunostaining.

These cut-off values were used because they correlate with the prognosis for GISTs based on histological preparations and also the data from the previous literature.[3,5,7,19]

Statistical analysis

Disease-free survival (DFS) was defined as the time from surgery to the first relapse of GISTs, the occurrence of a second primary tumor or death of any cause. DFS was calculated using the Kaplan-Meier method with a log-rank test. The time of the analysis for DFS was as of December 2007. The association of risk grades with the continuous variables of the clinicopathological parameters was explored with Student's t test. Correlation analyses of the expression of E2F1 or p53 with the clinical and pathologic variables were done using the χ^2-test. Univariate analysis of DFS was performed by the Kaplan-Meier method with using the log-rank test. We used the Cox proportional hazards model for the multivariate analysis of DFS. SPSS statistical software (version 12, Statistical Package for Social Science) was used for all the statistical analyses. A p-value <0.05 was considered to be statistically significant.

RESULTS

Clinicopathological features

According to the risk system suggested by Miettinen *et al*. (Table 1), 10 cases were classified as very low risk, 25 were classified as low risk, 9 were classified as intermediate risk and 28 were classified as high risk. As shown in Table 2, the 72 patients included 36 males and 36 females (mean age: 57.5 years, age range: 18 to 83 years).

The tumors were located within the esophagus (1 case), stomach (45 cases), small intestine (16 cases), colon (5 cases), rectum (3 cases), and extra-gastrointestinal sites (2 cases). The GISTs arising in extra-gastrointestinal (GI) sites were from the omen-

Table 1. Prognostic grouping and AFIP Risk grade of GIST by Miettinen *et al.*

Tumor parameters		Risk grade of malignant potential			
Size, cm	Mitotic rate per 50 HPFs	Gastric GISTs	No. of cases	Intestinal GISTs	No. of cases
≤2	≤5	Very low	9	Very low	1
3-5	≤5	Low	12	Low	3
6-10	≤5	Low	9	Intermediate	2
>10	≤5	Intermediate	1	High	2
≤2	>5	Low	1	High	1
3-5	>5	Intermediate	6	High	7
6-10	>5	High	3	High	3
>10	>5	High	5	High	7
Total			46		26

AFIP, Armed Forces Institute of Pathology; GIST, gastrointestinal stromal tumor.

tum and retroperitoneum and they were of a high-risk grade. Tumor location was significantly associated with the tumor risk grade ($p<0.001$, Spearman rho=0.542).

Fifty-seven tumors were the spindle type and 15 were the epithelioid type. Necrosis was present in 36 cases and hemorrhage was present in 43 cases. Necrosis was significantly associated with the risk grade ($p<0.000$).

Follow-up information was available for all 72 patients and the follow-up period ranged from 11 to 250 months. Metastasis was seen in 10 (13.8%) of the 72 patients, including 8 cases of liver metastasis, 1 case of omentum metastasis and 1 case of lymph node metastasis. Local recurrence or direct invasion to the mesentery was seen in 4 (5.5%) of the 72 patients. One case of gastric GISTs was accompanied with rectal GIST and all the tumors in this case were high-risk grade tumors. Five cases of gastric GISTs were synchronously accompanied with adenocarcinoma of the stomach (4 cases) and adenocarcinoma of the colon (1 case).

The 5-year survival rate was 75% and the 10-year survival rate was 72%. Overall, 52 of the 72 patients are still alive. Forty-nine of those patients (68%) are alive without any evidence of recurrence after the initial operation, 3 (4.1%) are alive with a recurrent tumor, 15 (20.8%) died of GIST (including 14 cases with a high risk grade GIST and 1 case with an intermediate risk grade GIST) and 5 died of other diseases.

Table 2. Clinicopathological data for 72 GISTs

	Very low+low risk (n=35)	Intermediate risk (n=9)	High risk (n=28)	p
Sex				0.280
Male (36)	16	3	17	
Female (36)	19	6	11	
Age (year) (18-83 years, mean: 57.5)	60.14	56.55	54.50	0.084
Location				<0.001*
Esophagus (1)+ stomach (45)	1+30	0+6	0+9	
Small intestine (16)	4	3	9	
Colon (5)+Rectum (3)	0+0	0+0	5+3	
Extra-GI site (2)	0	0	2	
Histologic type				0.402
Spindle (57)	29	8	20	
Epithelioid (15)	6	1	8	
Necrosis				<0.001*
Present (36)	9	5	22	
Absent (36)	26	4	6	
Hemorrhage				0.101
Present (43)	18	4	21	
Absent (29)	17	5	7	
Metastasis & recurrence (14)	1	1	12	<0.001*

*, statistically significant.
GIST, gastrointestinal stromal tumor.

Correlations of the E2F1 and p53 expressions with the clinicopathological variables

To evaluate the significance of the E2F1 and p53 expressions in the patients with GISTs, we compared the clinicopathologi-

Table 3. The correlation of E2F1, p53 with clinicopathologic parameters

	E2F1 (+) (n=18)	p	p53 (+) (n=22)	p
Age		0.683		0.386
≤60 (35)	8		9	
>60 (37)	10		13	
Tumor location		0.436		0.376
Esophagus (1)+ stomach (45)	14		11	
Small intestine (16)	2		6	
Colon+rectum (8)	2		4	
Extra-GI site (2)	0		1	
Tumor size		0.014*		0.044*
≤2 cm (13)	0		2	
2-5 cm (27)	12		8	
6-10 cm (19)	4		4	
>10 cm (13)	2		8	
Risk grade		0.192		0.003*
Low (35)	12		5	
Intermediate (9)	1		2	
High (28)	5		15	
Histologic type		0.241		0.372
Spindle (57)	16		16	
Epithelioid (15)	2		6	
Necrosis				
Present (36)	8	0.586	16	0.011*
Absent (36)	10		6	
Hemorrhage				
Present (43)	14	0.071	15	0.332
Absent (29)	4		7	
Mitotic counts		0.544		<0.001*
<5/50 HPF (37)	11		5	
5-10/50 HPF (8)	1		1	
>10/50 HPF (27)	6		16	
Ki-67 LI		0.753		0.140
≤4.92% (54)	13		14	
>4.92% (18)	5		8	
CD34		0.302		0.266
Positive (58)	16		16	
Negative (14)	2		6	
S-100		1.000		0.529
Positive (32)	8		11	
Negative (40)	10		11	
SMA		0.682		0.325
Positive (39)	9		10	
Negative (33)	9		12	

*, statistically significant.

cal characteristics of the patients whose samples displayed positive staining (Table 3). An E2F1 expression was observed in 18 patients (25%) and a p53 expression was observed in 22 patients (30.5%) (Fig. 1). Age was not significantly different between the E2F1 and p53 expression groups (p=0.683 and p=0.386).

There were no significant differences in tumor location among the groups with an E2F1 or a p53 expression (p=0.436 and p=0.376). An E2F1 expression was observed in 30.4% of the esophagus/stomach cases (14 of 46 cases), in 12.5% of the small intestine cases (2 of 16 cases), in 25% of the colon/rectum cases (2 of 8 cases) and in 0% of the extra-GI site GIST cases (0 of 2 cases). In addition, a p53 expression was observed in 24% of the esophagus/stomach cases (11 of 46 cases), in 37.5% of the small intestine cases (6 of 16 cases), in 50% of the colon/rectum cases (4 of 8 cases) and in 50% of the extra-GI site GIST cases (1 of 2 cases). Although E2F1 and p53 were expressed more strongly in the larger tumors (p=0.014 and p=0.044), an E2F1 expression was not related to tumor risk grade (p=0.192), while a p53 expression was associated with the tumor risk grade (p=0.003). There were no significant differences in the E2F1 expression or the p53 expression with regard to the histologic type of tumor (p=0.241, p=0.372). An E2F1 expression was observed in 28.1% of the spindle type cases (16 of 57 cases) and in 13.3% of the epithelioid type cases (2 of 15 cases). An E2F1 expression was not significantly associated with the mitotic counts (p=0.544) or with the KI-67 LI (p=0.753). By contrast, although p53 was expressed more often in the cases with an increased mitotic count (p<0.001), the p53 expression was not related to the KI-67 LI (p=0.140).

There were no significant differences in the E2F1 expression between the tumors with hemorrhage-present group and the tumors with hemorrhage-absent group (p=0.071), and nor were there significant differences of the p53 expression between the above mentioned groups (p=0.332). Although an E2F1 expression was not related to necrosis (p=0.586), a p53 expression showed a statistically significant association with necrosis (p=0.011).

The rate for a positive CD34 expression was 80% (58), the rate for a positive SMA expression was 54% (39) and that for S-100 was 44% (32). There was no statistical difference in the reactivity of these 3 antibodies in the cases with an E2F1 or p53 expression.

Correlation of the E2F1 expression with the p53 expression

The E2F1 and p53 labeling indices revealed that the cases with an elevated E2F1 expression exhibited elevated p53 expression and vice-versa. This correlation between E2F1 and p53 was confirmed statistically (Spearman rho=0.315 and p=0.007) (data not shown).

Relationship of the E2F1 and p53 expressions with disease-free survival

Kaplan-Meier survival curves were constructed to assess the prognostic significance of the E2F1 and p53 expressions (Fig. 2). The patients with E2F1-positive GISTs experienced significantly shorter DFS times than did the patients with E2F1-negative GISTs. The median DFS time of the patients in the E2F1-positive group was 107 months and that of the patients in the E2F1-negative group it was 160 months. These differences are statistically significant (p=0.039, log-rank test). The patients with p53-positive GISTs experienced significantly shorter DFS times than did the patients with p53-negative GISTs. The medi-

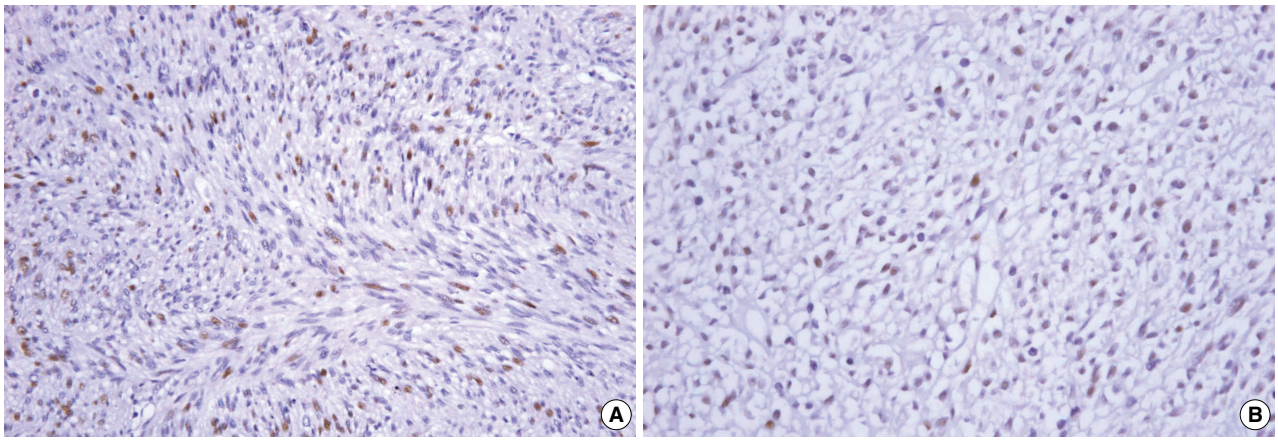

Fig. 1. Immunohistochemical expression of E2F1 (A) and p53 (B) in GISTs.

an DFS time of the patients in the p53-positive group was 57 months and that of the patients in the p53-negative group was 176 months. These differences are statistically significant (p< 0.0001, log-rank test).

Using forward stepwise Cox proportional hazards regression modeling, the following variables were tested for their influence on DFS: gender, age (≤60 vs >60), the risk grade (low/intermediate versus high), the histologic type (the spindle type versus the epithelioid type), the Ki-67 LI (≤4.92% vs >4.92%), the E2F1 expression and the p53 expression. Multivariate analysis revealed that the risk grade (p=0.003), the histologic type (p=0.001) and the expressions of E2F1 (p=0.001) and p53 (p= 0.004) were significant prognostic indicators of disease-free patient survival (Table 4). There were no significant correlation between the prognosis and the other clinicopathological features. An E2F1 expression was an independent predictor of poor DFS (hazard ratio=5.790, 95% confidence interval [CI]=2.100-15.965, p=0.001). In addition, a p53 expression was an independent predictor of poor DFS (hazard ratio=4.978, 95% CI= 1.664-14.894, p=0.004). Comparing the prognostic significance between the expressions of E2F1 and p53, the risk of mortality or disease-recurrence in the E2F1-positive patients was higher than in the p53-positive patients (hazard ratio=5.790, 4.978, respectively). Therefore, an E2F1 expression is a more powerful independent prognostic factor than a p53 expression in GISTs.

Table 4. Univariate and multivariate analyses of various parameters for DFS in 72 patients

Variables	n	Univariate p	Multivariate p	Hazard ratio (95% CI)
E2F1		0.039	0.001	5.790 (2.100-15.965)
Positive	18			
Negative	54			
P53		<0.0001	0.004	4.978 (1.664-14.894)
Positive	22			
Negative	50			
Risk grade		0.0006	0.003	5.436 (1.799-16.420)
Low, intermediate	44			
High	28			
Histology		0.0008	0.001	6.114 (2.198-17.004)
Spindle	57			
Epithelioid	15			

CI, confidence intervals; DFS, disease-free survival.

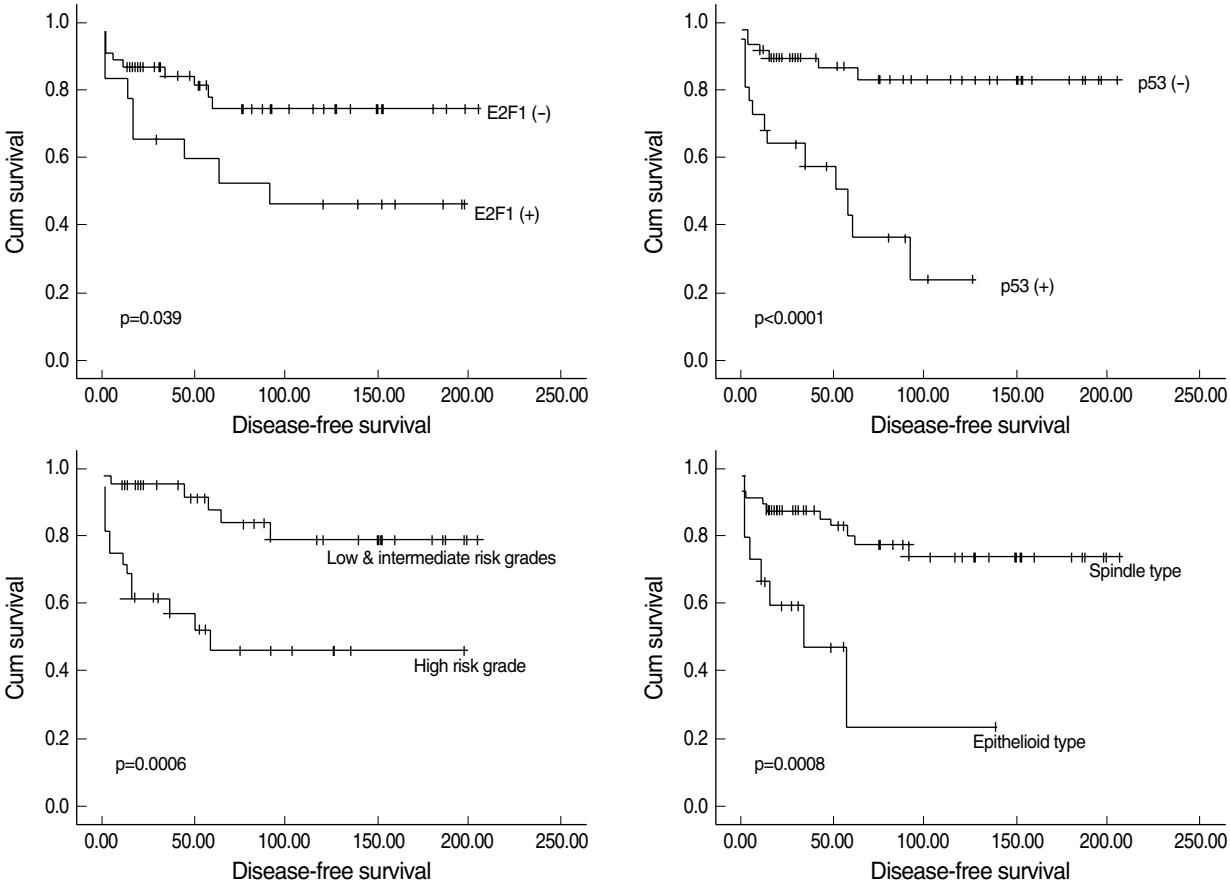

Fig. 2. Disease-free survival rate curves based on E2F1 (A), p53 (B), risk grade (C), and histologic type (D).

The median DFS time was 71 months. The median DFS time for the patients with a high-risk grade was 102 months, which was shorter than the 174 months for the patients with a low/intermediate risk grade (p=0.0006). On multivariate analysis, a high-risk grade had a more statistically significant association with DFS (p=0.003) than did the other risk grades, and a high-risk grade was an independent prognostic factor for a poor outcome (hazard ratio= 5.436, 95% CI=1.799-16.420).

The median DFS time for the patients with an epithelioid histologic type of GIST was 53 months, which is shorter than the 161 months for the patients with a spindle type GIST (p=0.0008). On multivariate analysis, the epithelioid type showed a statistically significant association with a shorter DFS period than did the spindle type, and the epithelioid type was the most powerful independent prognostic factor of a poor outcome (hazard ratio=6.114, 95% CI=2.198-17.004).

DISCUSSION

E2F1 is a transcriptional factor that has dual roles as an oncogene or a tumor suppressor depending on the various kinds of human cancers.[12,13] In normal physiology, inactive E2F1 is in complex with retinoblastoma protein (pRB).[12] When pRB is hyperphosphorylated during the transition from the G1 phase to the S phase, the E2F1 protein is released from the E2F1-pRB complex and the free E2F1 becomes available to transcriptionally activate various target genes that are required for DNA synthesis.[8] The transcriptional factor E2F1 is a relevant target for pRB in the cell proliferation pathway and the activation of E2F1 through the inactivation of pRB was found to contribute to tumor development in a murine model.[20] As E2F1 plays a distinct biologic role in the nucleus, it is localized in the nuclei of cancer cells. Only a few studies concerned with the correlation between E2F1 and GISTs have been published and they suggest that E2F1 acts as an oncogene in the tumorigenesis of GISTs.[7] In this study, a E2F1 expression was significantly correlated with a larger tumor size and shorter DFS (p=0.014, p=0.039), but it was not correlated with necrosis or hemorrhage (p=0.586, p=0.071). These results suggest that E2F1 may act as oncogene rather than as a tumor-suppressor in the tumorigenesis of GISTs. However, an E2F1 expression was not related to the mitotic counts or the Ki-67 LI, which are known to be related to proliferative activity (p=0.544, p=0.753). There may be that complex mechanisms are involved in the proliferation of GISTs.

p53 is one of the most well-documented tumor suppressor genes, and its immunohistochemical staining can reflect the alteration of the p53 gene. A previous study has shown that a p53 expression is correlated with a E2F1 expression and both the p53 and E2F1 expressions were significantly elevated together in malignant ovarian cancers.[15] Likewise in GISTs, a p53 expression is significantly correlated with an E2F1 expression (Spearman rho=0.315 and p=0.007). These results suggest that E2F1 is connected with the p53 pathway in the tumorigenesis of GISTs. Several studies have shown that E2F1 is related to p53.[21] In normal cells, E2F1 physiologically induces apoptosis via the p53 pathway.[9,21] In tumor cells, E2F1 can have a tumorigenic effect only on intact p53-containing cells, and when E2F is defect or damaged, then the tumor cells can be alternatively proliferated by the p53 pathway.[12,21]

A p53 expression was also significantly correlated with a high-risk grade, a larger tumor size, and shorter DFS in our study (p=0.003, 0.044, and <0.0001, respectively). A p53 expression showed strong correlation with tumor proliferation, which was similar to E2F1, in the present study. These results demonstrate both direct and indirect mutual control by p53 and E2F1 in the tumorigenesis of GISTs.[12] It has long been suggested that a p53 expression in GISTs could be an adverse prognostic factor.[22] On comparing the prognostic significance between E2F1 and p53, the risk of mortality or disease-recurrence for the E2F1-positive patients was higher than that for the p53-positive patients (hazard ratio=5.790, 4.978, respectively). Therefore, an E2F1 expression is a more powerful independent prognostic factor than a p53 expression in GISTs.

The histologic type was significantly correlated to both the risk grade and DFS. This suggests that the epithelioid type is a powerful independent prognostic factor for predicting a poor DFS outcome (hazard ratio=6.114, 95% CI=2.198-17.004).

We found no correlation for the tumor location as a prognostic factor. However, the prognostic outcome of extra-GI GISTs (EGISTs) was interesting among the various GIST sites. The frequency of EGIST is low (approximately 1% of all GISTs).[23] We had two cases of EGIST of the omentum and retroperitoneum with each follow-up period being 38 months and 136 months, respectively. These patients were both females and they were 67 years of age at the time of diagnosis. Although both were classified into a high risk grade, they are healthy now and they showed favorable outcomes. The results of the previous studies have demonstrated that EGISTs of the omentum and retroperitoneum had a good prognosis and this corresponded with our results.[24] It seems impracticable that the criteria for small intestinal GISTs in the AFIP system should be applied to the

EGISTs of the omentum and retroperitoneum.

The immunopositivity rate for CD34, S-100 and SMA was 80%, 44%, and 54%, respectively. Some review articles have showed that 80% to 85% of gastric GISTs, 50% of small intestinal GISTs, and 95% to 100% of esophageal and rectal GISTs were positive for CD34.[23] Among gastric GISTs, the spindle types were usually CD34-positive (>90%), whereas the epithelioid ones were more variably CD34-positive (58%). Twenty percent of gastric GISTs and 35% of small intestinal GISTs were SMA-positive.

Less than 1% of gastric GISTs and 14% to 50% of the small intestinal GISTs were S-100-positive. The discrepancies in the percentages of positivity may be due to the differences of the employed antibody and the tissue fixation methods and the variations in the staining methods among the different studies. In the previous large studies of GISTs, SMA positivity was seen as a favorable prognostic factor for both gastric and small intestinal GISTs.[23] S-100 positivity was previously reported as an adverse prognostic factor for gastric GISTs.[23] In our study, there was no statistically significant difference between CD34, SMA, or S-100 in regards to DFS (p=0.321, 0.264, and 0.918, respectively) (data not shown).

We did not find any prognostic correlation with the KI-67 LI and the mitotic counts when they were categorized by each cut-off value (4.96%, and <5/50HPF, 5-10/50 HPF and >10/50 HPF) (p=0.908, p=0.730) (data not shown). In addition, tumor size was not significantly correlated with the prognostic outcome when the tumor size was categorized into <2 cm, 2-5 cm, 5-10 cm, and >10 cm (p=0.458) (data not shown). A high-risk grade that included both the tumor size and the mitotic counts was strongly correlated with a poor DFS outcome (hazard ratio=5.436, 95% CI=1.799-16.420).

In conclusion, we have found a significant correlation between a high-risk grade (using the AFIP system) and a poor prognosis according to the 18 year follow-up data at our hospital. Furthermore, we recommend E2F1 as being a potential biologic marker that is predictive of a worse prognosis for GISTs. p53 is also a useful prognostic marker, which is consistent with the previous literature. Further studies are needed to molecularly analyze E2F1 and p53 and the tumor/biochemical pathways they are involved in.

ACKNOWLEDGEMENT

The authors would like to thank Moon Sook Oh for her excellent technical assistance.

REFERENCES

1. Thomas RM, Sobin LH. Gastrointestinal cancer; incidence and prognosis by histologic type, SEER population-based data 1973-1987. Cancer 1995; 75: 154-70.
2. Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 1998; 152: 1259-69.
3. Miettinen M, Lasota J. Gastrointestinal stromal tumors: review on morphology, molecular pathology, prognosis and differential diagnosis. Arch Pathol Lab Med 2006; 130: 1466-78.
4. Fletcher CD, Berman JJ, Corless C, *et al.* Diagnosis of gastrointestinal stromal tumors: a consensus approach. Hum Pathol 2002; 33: 459-65.
5. Nakamura N, Yamamoto H, Tao T, *et al.* Prognostic significance of expressions of cell-cycle regulatory proteins in gastrointestinal stromal tumor and the relevance of the risk grade. Hum Pathol 2005; 36: 828-37.
6. Dimova DK, Dyson NJ. The E2F transcriptional network: old acquaintances with new faces. Oncogene 2005; 24: 2810-26.
7. Haller F, Guanawan B, von Heydebreck A, *et al.* Prognostic role of E2F1 and members of the CDKN2A network in gastrointestinal stromal tumors. Clin Cancer Res 2005; 11: 6589-97.
8. Sherr CJ. The INK4a/ARF network in tumour suppression. Nat Rev Mol Cell Biol 2001; 2: 731-7.
9. Stevens C, La Thangue NB. E2F and cell cycle control: a double-edged sword. Arch Biochem Biophys 2003; 412: 157-69.
10. Attwooll C, Lazzerini Denchi E, Helin K. The E2F Family: specific functions and overlapping interests. EMBO J 2004; 23: 4709-16.
11. LaThangue NB. The yin and yang of E2F-1: balancing life and death. Nat Cell Biol 2003; 5: 587-9.
12. Eymin B, Gazzeri S, Brambilla C, Brambilla E. Distinct pattern of E2F1 expression in humnan lung tumours: E2F1 is up-regulated in small cell lung carcinoma. Oncogene 2001; 20: 1678-87.
13. Zhang SY, Liu SC, Al-Saleem LF, *et al.* E2F1: a proliferative marker of breast neoplasia. Cancer Epidemiol Biomarkers Prev 2000; 9: 395-401.
14. Saiz AD, Olvera M, Rezk S, Florentine BA, McCourty A, Brynes RK. Immunohistochemical expression of cyclin D1, E2F1, and Ki-67 in benign and malignant thyroid lesions. J Pathol 2002; 198: 157-62.
15. Kim JY, Kim HW, Choi KU, *et al.* Alteration of G1/S Cell Cycle Regulatory Proteins in Ovarian Epithelial Tumors. Korean J Pathol 2006; 40: 274-81.

16. Zacharators P, Kotsinas A, Evangelou K, *et al.* Distinct expression patterns of the transcription factor E2F1 in relation to tumour growth parameters in common human carcinomas. J Pathol 2004; 203: 744-53.
17. Rabbani F, Richon VM, Orlow I, *et al.* Prognostic significance of transcription factor E2F-1 in bladder cancer: genotypic and phenotypic characterization. J Natl Cancer Inst 1999; 91: 874-81.
18. Kononen J, Bubendorf L, Kallioniemi A, *et al*. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4: 844-7.
19. Meara RS, Cangiarella J, Simsir A, Horton D, Eltoum I, Chhieng DC. Prediction of aggressiveness of gastrointestinal stromal tumours based on immunostaining with bcl-2, Ki-67 and p53. Cytopathology 2007; 18: 283-9.
20. Dyson N. The regulation of E2F by pRB-family members. Genes Dev 1998; 12: 2245-62.
21. Sharma N, Timmers C, Trikha P, *et al.* Control of the p53-p21^{CIP1} Axis by E2f1, E2f2, and E2f3 Is Essential for G1/S Progression and Cellular Transformation. J Biol Chem 2006; 281: 36124-131.
22. Al-Bozom IA. p53 overexpresion in gastrointestinal stromal tumors. Pathol Int 2001; 51: 519-23.
23. Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. Semin Diag Pathol 2006; 23: 70-83.
24. Reth JD, Goldblum JR, Lyles RH, *et al.* Extragastrointestinal (soft tissue) stromal tumors. An analysis of 48 cases with emphasis on histological predictors of outcome. Mod Pathol 2000; 13: 577-85.