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p27 expression and proliferation in gastrointestinal stromaltumors

Gastrointestinal stromal tümörlerde p27 ekspresyonu ve hücre çoğalması

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Background/aims: *The p27 is a cyclin-dependent kinase inhibitor which plays a pivotal role in cell proliferation during development and tumorigenesis. Recently, it was shown that low expression of p27 tends to occur in more aggressive neoplasms. In gastrointestinal stromal tumors p27 expression and its correlation with proliferation remain to be elucidated. The aim of the present study was to investigate the expression of p27 and proliferation in gastrointestinal stromal tumors.* ***Methods:*** *p27 and Ki-67 immunostained tissue sections from 45 gastrointestinal stromal tumors (benign, borderline and malignant) were subjected to quantitative analysis, and p27 labelling index and Ki-67 labelling index were obtained for each case.* **Results:** *The mean values of Ki-67 labelling index in the three groups were significantly different (benign, 4.53%; borderline, 9.82%; and malignant, 15.35%) ($p<0.05$). p27 labelling index was decreased in malignant tumors (10.46%) compared with benign (36.56%) and borderline tumors (21.36%) ($p<0.05$). Moreover, in each group p27 labelling index exhibited a significant inverse relation to p67 labelling index ($p<0.001$).* ***Conclusion:*** *Our results suggest that decreased levels of p27 labelling index could predict the malignant behavior of gastrointestinal stromal tumors and might be associated with tumor cell proliferative activity.*

Key words: Gastrointestinal stromal tumors, proliferation, p27, Ki-67.

Amaç: *p27 gelişim ve tümörogenez boyunca hücre çoğalmasında önemli rol oynayan, sikline bağımlı kinaz inhibitörüdür. Geçmişte düşük p27 ekspresyonunun daha agresif neoplazilerde bulunmaya eğilimli olduğu gösterilmiştir. Gastrointestinal stromal tümörlerde ise p27 ekspresyonu ve p27 ekspresyonunun proliferasyon ile ilişkisi henüz araştırılmamıştır. Bu çalışmanın amacı gastrointestinal stromal tümörlerde p27 ekspresyonu ve proliferasyonu araştırmaktır.* ***Yöntem:*** *Her bir olguya ait p27 ve Ki-67 ile immünohistokimyasal olarak boyanmış doku kesitlerinde kantitatif olarak p27 bağlannma endeksi (p27LI) ve Ki-67 bağlanma endeksi (KLI) belirlendi.* ***Bulgular:*** *Uç grubun KLİ'i arasında anlamlı bir fark olduğu görüldü (benign, 4.53%; sınır olgu, 9,82%; ve malign,15.35 %)($p<0.05$). p27LI'i benign (36.56%) ve sınır olgular (21.36%) ile karşılaştırıldığında malign olgularda daha düşüktü (10.46%) ($p<0.05$). Ek olarak, her bir grupta p27LI ile KLI arasında anlamlı ve ters bir korelasyon olduğu görüldü ($p<0.05$).* ***Sonuç:*** *Sonuçlarımız, düşük p27LI'nin gastrointestinal stromal tümörler'in malign davranışını belirleyebileceğini ve tümör hücrelerinin proliferative aktiviteleri ile ilişkili olabileceğini düşündürmektedir.*

Anahtar kelimeler : Gastrointestinal stromal tümör, proliferasyon, p27, Ki-67

INTRODUCTION

Gastrointestinal stromal tumors (GIST) are a heterogeneous group of neoplasms, and their biological behavior is a persistent source of controversy. In recent studies, many parameters have been reported as useful to predict the prognosis of these tumors, and measurement of proliferative index is amongst them (1-3). Although there is some disagreement, recent studies have proven the impact of high proliferative index on the malignant potential of GIST (1-6).

The cell cycle is a complex process governed by a family of cyclins and cyclin-dependent kinases (CDK). The cellular D-type cyclins contribute to cell cycle control by forming complexes with CDK (7, 8). The activation of the CDK/cyclin complex is controlled by CDK in hibitors, which regulate cell cycle (8). p27, a major inhibitor of the CDK, is a nuclear phosphoprotein belonging to the Kip family of CDK inhibitors (9-13). In physiological conditions the expression of p27 is highest in quiescent cells and declines when cells proliferate in response to mitogenic signals such as growth factors and cytokines, suggesting that it also plays a role in maintaining cells in GO (14-16). Overexpression of p27 protein in mammalian cells induces a Gl block of the cell cycle. p27 may act as

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a tumor suppressor, and several reports in solid neoplasm, including gastrointestinal system, suggest that loss or low level of p27 expression is associated with aggressive behavior and poor prognosis (17-19). Moreover, the inverse relationship between p27 expression and proliferation observed physiologically in normal tissues is also related with aggressive behavior and poor prognosis in many organ tumors (20-21).

Although in GIST the relationship between cell proliferation and tumor behavior have been investigated by some researchers (1-6), neither p27 expression nor its correlation with proliferation has been delineated. Therefore, the aim of the present study was to investigate p27 labelling index (p27LI) in GIST and its relationship between tumor behavior and proliferation.

MATERIALS AND METHODS

Forty-five cases of GIST were included in this study. All tumors were primarily treated by resection at the Department of Surgery, Akdeniz University, Antalya from 1989 to 1997. The median age of the 25 men was 51 years (range 39-64 years) at the time of operation, and that of the 20 women, 49 years (range 32-61 years).

Four micrometer thick hematoxylin and eosin stained tissue sections from the surgical specimens fixed in 10% formalin and embedded in paraffin were reviewed, and tumors were divided in three groups: benign (mitotic index [MI]< 5/50 high-power fields [HPF] and size < 5 cm), borderline (MI < 5/50 HPF and size > 5 cm), and malignant (MI > 5/50 HPF, irrespective of size).

Distribution of clinicopathologic characteristics of each group are summarized in (Table 1).

Four micrometer thick hematoxylin and eosin stained tissue sections from the surgical specimens fixed in 10% formalin and embedded in paraffin were reviewed and representative tissue blocks were selected. For tumor phenotyping antibodies to vimentin (V9, mouse monoclonal, dilution: 1/50, Dako, Denmark), smooth muscle actin (1A4, mouse monoclonal, dilution: 1/50, Dako, Denmark), desmin (D33, Mouse monoclonal, dilution: 1/50, Dako, Denmark), S-100 protein (polyclonal rabbit, dilution: 1/50, Dako, Denmark), neuron-specific enolase (BBS/NC/VT-H14, mouse monoclonal, dilution: 1/50, Dako, Denmark), CD34 (QBEnd 10, mouse monoclonal, dilution: 1/50, Dako, Denmark) and CD117 (polyclonal rabbit, dilution: 1/50, Dako, Denmark) were applied. Monoclonal antibodies to Ki-67 (MIB-1, dilution 1: 80, Immunotech, Marseille, France) and p27 (SX53G8, mouse monoclonal, dilution: 1/50, Dako, Denmark) were used for quantitative assessment of Ki-67 labelling index (KLI) and p27LI, respectively. Sections were deparaffinized and heated in a microwave oven for 10 minutes to retrieve antigens. Slides were immunostained with primary antibodies by the avidin-biotin immunoperoxidase technique. Finally, all slides were treated with DAB reagent to develop color and were counterstained with hematoxylin.

For cytoplasmic antigens, whole tumor surface was evaluated on at least three sections. Internal positive controls were blood vessels (vimentin, desmin, SMA and CD34), nerve fascicles (S-100), mast cells (CD117), neuroendocrine cells (NSE) and mucosal cells (Ki-67 and p27).

Slides were interpreted for KLI and p27LI by a pathologist who had no knowledge about the clinicopathologic data.

Assessment of KLI and p27LI was performed at the highest proliferative areas. In each case, positive and negative nuclei were counted at x400 magnification. KLI and p27 were calculated as the percentage of positive tumor cells relative to the total number of cells counted.

Chi-square was used to compare frequencies. The difference in numerical data between the three groups was analyzed by using Student's t test. Correlations among various parameters were tested by calculating Spearman's correlation coefficient. A significance level of 0.05 was used throughout the analysis.

RESULTS

The most prevalent antigen was CD117, being expressed in all tumors, followed by CD34, which was positive in 39 cases. Reactivity for SMA was seen in 24 cases and four of them were positive for desmin. Neural differentiation, as assessed by the expression of either S-100 protein or NSE, was present in 31 cases. Eleven tumors co-expressed muscular and neural antigens as well as CD34 in nine cases.

In normal mucosa, cells expressing p27 protein were present only in the superficial two-thirds of the epithelium and Ki-67 staining was noted in the lowest one-third of crypts in all cases. In normal mucosa and GIST, Ki-67 and p27 immunos-

Table I: Distribution of clinicopathologic characteristics, phenotypic antigen expression, KLI and p27LI in each group

	BENIGN *(n: 15)*	BORDERLINE *(n: 15)*	MALIGNANT *(n: 15)*
Age	52	48	53
Gender			
Male	9	7	8
Female	6	8	7
Location			
Stomach	8	5	6
Intestine	5	6	8
Colon	2	4	1
Cell type			
Spindle	8	9	10
Epithelioid	7	6	5
Immunophenotyping			
CD117			
Positive	15	15	15
Negative	-	-	-
CD34			
Positive	14	13	12
Negative	1	2	3
SMA			
Positive	10	8	6
Negative	5	7	9
Desmin			
Positive	2	1	1
Negative	13	14	14
S-100			
Positive	9	5	4
Negative	6	10	11
NSE			
Positive	5	6	5
Negative	10	9	10
KLI			
Meant SD*t	4.53 ± 2.17	9.82 ± 3.08	15.35 ± 3.41
Median	4.3	9.5	15
Ranges	0.9-9.82		
P27LI		4.9-16.5	9.3-21.3
Mean± SD*f	36.56+13.25	21.36±10.03	10.46±12.74
Median	31	19.8	19.6
Ranges	26.4-75.3	0-46.3	0-35.9

*Standard deviation, †p<0.05
KLI: Ki-67 labelling index; p27 labelling index.

taining was diffuse or granular and confined to the nucleus (Figures 1 and 2). The mean values of KLI in the three groups were significantly different (benign, 4.53%; borderline, 9.82%; and malignant, 15.35%) (p<0.05) (Figure 3) (Table 1).

p27LI of the benign tumors was found to be greater (36.56%) than that of p27LI of borderline (21.36%) and malignant (10.46%) tumors, respectively. The difference between p27LI of the three groups was significant (p<0.05) (Figure 3) (Table 1).

No correlation between KLI and p27LI and clinicopathologic factors was detected.

The relationship between KLI and p27LI was evaluated in each group separately. Spearman's correlation test revealed a strong inverse correlation between KLI and p27LI in benign (r=-0.86), borderline (r=-0.92) and malignant (r=-0.71) tumors (p<0.001).

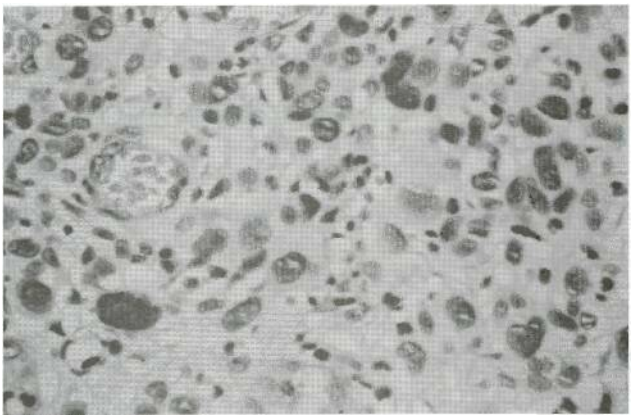

Figure 1: Ki-67 immunoreactivity in a case of GIST (MIB-1 monoclonal antibody, counterstained with hematoxylin, x400).

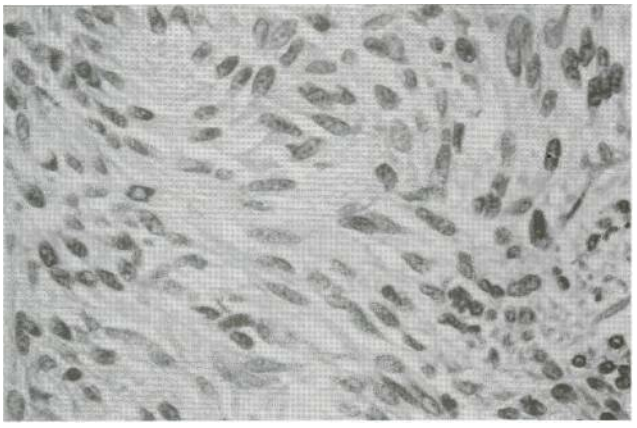

Figure 2 p27 immunoreactivity in a case of GIST (SX53G8 monoclonal antibody, counterstained with hematoxylin, x400).

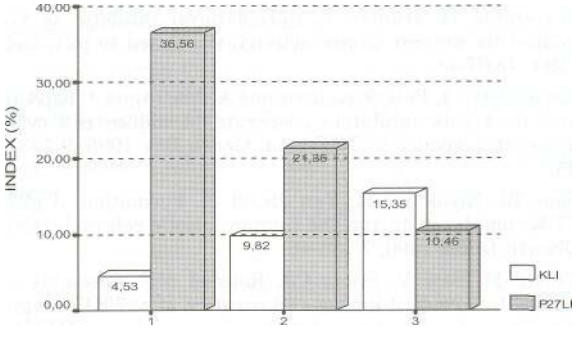

Figure 3: Mean KLI and p27LI indices in three groups (1: benign; 2: borderline; 3: malignant).

DISCUSSION

It is well established that cell proliferative kinetics are important to predict the biological behavior of many organ tumors. Many studies have proven that the evaluation of proliferative indices is a powerful criterion in distinguishing benign from malignant GIST (1-6). Our results support this data because proliferative indices evaluated by means of Ki-67 monoclonal antibody showed significant differences between the three groups. Nevertheless, regarding numerical values on the assessment of proliferative indices in GIST, data did not reach an agreement. It has been noted that the use of different antibodies to define proliferating cells is an important limitation to predict the exact proliferative rate of many organ tumors (22). In GIST, some studies used proliferating cell nuclear antigen to assess the cell growth fraction, but the significance of its reactivity is questionable (2-4, 22). We therefore applied a more reliable marker of cell proliferation, Ki-67 monoclonal antibody, to highlight proliferating cells, and the mean KLI of the whole group was 9.3%. In recent studies, mean values of KLI in GIST show differences between studies, ranging from 4% to 22% (3-6). We suggest that different methodologies in the assessment of proliferation and interobserver variations could contribute to this discordance between numerical values (4-5). However, these do not exclude the significance of our findings that showed a close correlation between KLI and malignant behavior in GIST. Our data support that evaluation of proliferation by means of KLI could be valuable in predicting malignant potential of GIST.

Cell proliferation is regulated by extracellular factors, which influence primarily during the prereplicative (Gl) phase of the cell cycle (23). These factors with their stimulatory or inhibitory signals converge on cell cycle regulatory proteins that predict whether cells progress through Gl phase (23). The major components of these cell cycle control proteins are cyclins and CDK. Their activity is regulated by intracellular proteins, including CDK inhibitors (8). p27 is a CDK inhibitor of the Gl-to-S cell cycle progression which suppresses the kinase activity of cyclin/CDK complex (9-13). The protein expression of p27 is often deregulated in human tumors. Recent studies have shown that absent or low p27 protein expression is a powerful negative prognostic marker in many organ tumors (17-19). In some studies, p27 expression was also

evaluated in soft tissue tumors. In synovial sarcomas, p27LI was not correlated with prognosis (24). However, a low p27LI was found to be correlated with malignant potential of malignant peripheral nerve sheath tumors (25). To our knowledge, p27 expression has not been evaluated in GIST. Our study demonstrated that benign GISTs show higher p27LI. However a remarkable decrease in p27LI in borderline and malignant groups was observed, indicating that the underexpression of p27 may play an important role in malignant progression in GIST. This data supports the previous observations on deregulated expression of p27 in malignant tumors and led us to speculate that evaluation of p27LI in GIST could be one of the important parameters to estimate the aggressive behavior of these tumors (17-19, 23).

Recently, high proliferative activity, as measured by Ki-67 antigen, has been shown to correlate with reduced p27 expression in lymphomas and endocrine tumors (20-21). However, the relation between KLI and p27LI was not previously analyzed in GIST. Therefore, we performed the study to also investigate the association of proliferative activity (as measured by the expression of Ki-67) with alterations of p27LI in these tumors. Our results revealed that p27LI and KLI had a strong and significant inverse correlation in each group ($p < 0.001$). This may indicate that p27 protein contributes to, or reflects, increased cell proliferation in GIST.

Unfortunately in the present study no survival data was available for a majority of cases. We propose that further studies are needed to establish the relationship between p27LI and prognosis in GIST.

Although only a limited number of cases has been included in this study, this is the first attempt to evaluate p27LI and its association with proliferation in GIST. The present study demonstrates that decreased p27LI correlates with malignant potential of GIST. We also showed that p27LI is inversely related to increased proliferative activity measured by KLI in these tumors. Although other mechanisms that influence the changes in proliferation in the GIST cannot be excluded, the results of our study support that p27 has an essential role in the proliferation of cells in these tumors.

Further investigations in larger series with follow-up information might provide not only a better understanding of the role of p27 expression in malignant transformation in GIST, but also help in determining the predictive value of p27LI in prognosis of patients with GIST.

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