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INVESTIGATION OF RELATIONSHIPS BETWEEN KI-67 SCORE, DNA INDEX, AND HISTOLOGIC GRADE IN SOFT TISSUE SARCOMAS

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Proliferative activity of soft tissue sarcomas (STS) in 31 cases was estimated by histologic grading, mitotic count, DNA analysis by flow cytometry, and immunohistochemical procedures with monoclonal antibody Ki-67. Aneuploid was found in 12 of 16 cases (75.0%) with Grade 3, and in 4 of 15 cases (26.7%) with Grade 1, 2 (P=0.0121). Tumors with more than 100 Ki-67 positive tumor cells per 10 high power fields (HPF) had a higher rate of aneuploid (81.3%) than those with less than 100 Ki-67 per 10 HPF (26.7%) (P=0.0038). There were significant correlations between Grade and DI (r=0.4901, P=0.0051), Grade and Ki-67 (r=0.4636, P=0.0086), Ki-67 and DI (r=0.6368, P=0.0001). The results indicate that DI and reactivity of tumor cells to Ki-67 may reflect proliferative activity and be helpful for clinicians to judge the biological behaviour of tumors more accurately and objectively. Supplementary to the grading of STS, DI and Ki-67 score could be useful as prognostic parameters for clinical investigation of multimodality therapy for individual patients.

Key words: Soft tissue sarcomas, Ki-67 antibody, Flow cytometry, Histologic grade.

Investigations of cell kinetics have established that the assessment of cell proliferation may be an important prognostic index for some tumors. In soft tissue sarcomas (STS), the most important factor affecting patient survival is histologic grade which has been used by pathologists to estimate the proliferative activity of the tumor and has proposed valuable information for clinicians to choose treatment remedy for patients.[1,2] Recently, Ki-67 expression by immunohistologic staining and DNA analysis by flow cytometry have been reported to have correlation with proliferative activity in a number of neoplasms, including carcinomas of breast, lung, colon, as well as lymphomas and a mixed group of sarcomas.[3,4] The purpose of this study was to clarify the relationships between mitotic count, histologic grade, DNA index (DI), and Ki-67 score with an attempt to help clinicians judge the biological behaviour of STS more accurately.

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MATERIALS AND METHODS

Tumor Selection

Thirty-one patients with primary or recurrent STS located in extremities, trunk, and retroperitoneum were selected for the current study, they were all subjected to surgical resection of the lesions without receiving radiotherapy or chemotherapy three months before surgery. There were 21 male and 10 female patients with a mean age of 46 years (range from 14 to 77 years) and a mean longest tumor size of 10.4 cm (range from 0.8 to 30 cm). Fresh tumor specimens

were obtained from surgical resection for histopathologic assessment of diagnosis, DNA flow cytometric analysis, and Ki-67 determination. The histologic types of sarcomas are listed in Table 1.

Table 1. Histologic types of 31 patients with soft tissue sarcomas

Histologic types	No. (%)
Rhabdomyosarcoma	5 (16.1)
Malignant schwannoma	5 (16.1)
Fibrosarcoma	5 (16.1)
Malignant fibrous histocytoma	4 (12.9)
Liposarcoma	4 (12.9)
Leiomyosarcoma	2 (6.5)
Undifferentiated sarcoma	2 (6.5)
Hemangiopericytoma	1 (3.2)
Synovial sarcoma	1 (3.2)
Unclassified	2 (6.5)
Total	31 (100)

Mitotic Count and Histologic Grading

Sections, cut at 5 μm, were stained with hematoxylin and eosion, and the number of mitosis per ten high power fields (HPF, magnification 400) was counted. The histologic grades were determined according to the criteria of Russell.[5]

Flow Cytometric Analysis

With EPICS V flow cytometry (Coulter Co., USA), a mean number of 10000 cells of every single-cell suspension sample obtained mechanically and enzymatically from fresh tumor tissue were analyzed for measurement of DNA content and DI. The DI was calculated from the DNA histogram as the ratio of G1/G0 peak of tumor cells to the G1/G0 peak of normal controlled diploid cells. In this study, lesions were classified as aneuploid if the DI was greater than 1.1 and as diploid if the DI was equal to or less than 1.1. The coefficients of variation (CV) ranged from 3.7% to 8.2% (mean=5.1%).

Ki-67 Immunohistochemical Staining

For immunohistochemical procedures, we used avidin-biotin-peroxidase complex (ABC) method.[6] Cryostat sections, cut at 5 μm, were air-dried at room temperature, fixed in acetone for 5 minutes, rinsed in TBS (0.05 M) and air-dried. The Ki-67 monoclonal antibody (Dakopatts, Denmark) was diluted in TBS (1:40), sections were incubated for 60 minutes in a moist chamber at room temperature. Subsequently, the sections were rinsed in TBS, incubated with biotinylated horse anti-mouse IgG (Vector, USA diluted in TBS, 1:200) for 30 minutes, rinsed, incubated with ABC complex (Vector, USA. diluted in TBS, 1:100) for 60 minutes, and rinsed again in TBS. Sections were stained for 5–10 minutes with freshly prepared diaminobenzidine (DAB) solution and counterstained with methyl green.

RESULTS

Mitotic Count and Grade

The mean mitotic score ranged from 0 to 13/10 HPF (mean, 3.4±3.1/10 HPF). The sarcomas were graded as Grade 1 (G1) in 7 patients, Grade 2 (G2) in 8, and Grade 3 (G3) in 16; their mean mitotic score was 1.0/10 HPF, 3.1/10 HPF, and 4.6/10 HPF, respectively.

DNA Ploidy and DI

Aneuploid was found in 17 tumors (54.8%) and dipoid in 14 (45.2%). DI varied from 0.67 to 2.4 (mean, 1.3±0.4). The mean DI was 1.0 in sarcomas with G1, 1.3 with G2, and 1.5 with G3. DNA ploidy had no significantly different distribution considering sex, age, tumor size, primary or recurrent tumor, and mitotic score. However, aneuploid was found higher in lesions with G3 (75.0%) than in lesions with G1, 2 (26.7%) and the difference was significant (Table 2).

Ki-67 Score

Ki-67-positive tumor cells could be clearly detected by their characteristic diffuse granular or globular nuclear staining. The percentage of stained cells often varied from area to area. In some cases, Ki-67 staining was restricted to certain architectural components of the tumor. The area containing the largest number of Ki-67-positive cells were selected, and the numbers of positive cells counted in 10 HPF chosen at random. Twenty-one sacomas (67.7%)

were positively stained. The Ki-67 scores ranged from 0 to 504/10 HPF (mean, 113/10 HPF). In lesions with G1, G2, and G3, the mean Ki-67 scores were 23.7/10 HPF, 90.3/10 HPF, and 172.8/10 HPF, respectively. Aneuploid was found in 13 of 16 cases (81.3%) with Ki-67 score of more than 100/10 HPF, compared with 4 of 15 cases (26.7%) with Ki-67 scores of less than 100/10 HPF (P=0.0038).

Table 2. Ploidy distribution of various factors in 31 patients with soft tissue sarcomas

Factors	Aneupoid	Diploid	Aneupoid
Sex			
Male (21)	11 (52.4)	10 (47.6)	0.7207
Female (10)	6 (60.0)	4 (40.0)	
Age			
≥50 years (14)	7 (50.0)	7 (50.0)	0.7247
<50 years (17)	10 (58.8)	7 (41.2)	
Tumor size			
≥10 cm (15)	8 (53.3)	7 (46.7)	1.0000
<10 cm (16)	9 (56.3)	7 (43.7)	
Primary or recurrent			
Primary (12)	6 (50.0)	6 (50.0)	0.7241
Recurrent (19)	11 (57.9)	8 (42.1)	
Mitotic count			
≥3/10 HPF (13)	8 (61.5)	5 (38.5)	0.7168
<3/10 HPF (18)	9 (50.0)	9 (50.0)	
Histologic grade			
G1, 2 (15)	4 (26.7)	11 (73.3)	0.0121
G3 (16)	12 (75.0)	4 (25.0)	
Ki-67 score			
≥100/10 HPF (16)	13 (81.3)	3 (18.7)	0.0038
<100/10 HPF (15)	4 (26.7)	11 (73.3)	

Correlations

The relationships between mitotic score, histologic grade, DI, and Ki-67 score wee evaluated by Pearson's rank correlation test (Table 3). Ki-67 score had statistically significant correlation with histologic grade (Figure 1) and DI (Figure 2). Histologic grade significantly correlated with mitotic score and DI. However, mitotic score had no positive correlation with DI and Ki-67 score.

DISCUSSION

In 1977, a clinical and pathologic staging system for STS was proposed by Russell et al.[5] In this system, histologic grade, tumor size, and extend of the tumors were factors for staging of the tumor. Histologic grade was mainly determined according to frequencies of mitosis. Meanwhile, the frequencies of mitosis did not necessarily correlate with grade of malignancy in some cases: STS of small round cell type such as Ewing's sarcomas and undifferentiated sarcomas were proved to be high-grade tumors but usually showed low mitotic activity.[7] Therefore, it is necessary to use more accurate and objective methods to evaluate the cell proliferation in STS to estimate the prognosis in STS and help clinicians treat individual patients.

Complementary to conventional histologic assessment, DNA content or DI detected by flow cytometry has been shown as a parameter to make a distinction between benign and malignant tumors and reflect the proliferative activity of malignancy.[3] Kreicbergs, et al. found a relationship between tumor grade and DI in STS, and indicated that the Grade 3

sarcomas with aneuploid had a poorer prognosis than the same grade with diploid.[8] Our results support their observations, i.e., aneuploid was associated with higher grades of malignancy, and ploidy or DI may reflect the degree of malignancy and be an important prognostic factor in STS.

Table 3. Result of spearman's rank correlation test for variables of mitotic count, histologic grade, DI, and Ki-67 score

Variables		Grade	Mitosis	DI
Ki-67	*r*	0.4636	0.1849	0.6368
	P	0.0086	0.3194	0.0001
Grade	*r*		0.5220	0.4901
	P		0.0026	0.0051
Mitosis	*r*			0.1210
	P			0.5166

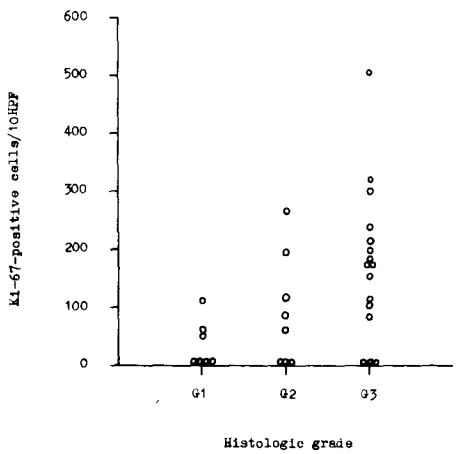

Fig. 1. Ki-67 score correlated well with histologic grade (r=0.4636, P=0.0086).

Using immunohistochemical staining with Ki-67 monoclonal antibody to measure cell proliferation was reported by many authors.[4] The Ki-67 antibody, which reacts with a nuclear antigen expressed in all phases of the cell cycle except G0, has been shown to be more reliable and available as a simple histologic marker of proliferative activity in cells of various tissues and malignant neoplasms.[4,9] In a study of 34 cases with STS, Ueda, et al. reported that Ki-67 index positively correlated with histologic grade and the Ki-67 low index group (less than 50/10 HPF) showed more favorable prognosis than the high index group (more than 50/10 HPF) (P<0.005).[7] In our study, the relationships between histologic grade, DI, and Ki-67 were found to be significant. The prognostic value of these parameters will be analyzed when follow-up times of all patients in this study are available.

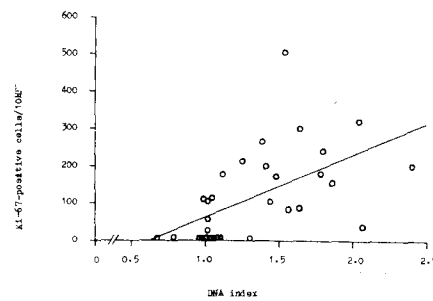

Fig. 2. Ki-67 score correlated well with DNA index (r=0.6368, P=0.0001).

Because cells in M phase spend relatively shorter time in cell cycle, it is less reliable to judge the cell proliferation by counting mitosis without taking account of cellularity, degree of tumor necrosis, and clinical characteristics. The mitotic count is not a standardized method and is often not reproducible even among the experienced pathologists. Therefore, the mitotic count cannot be regarded as an independent parameter of cell proliferation and the mitotic activity of a tumor can only be interpreted in the context of the other pathological and clinical findings.[10] We consider that instead of mitosis the reactivity of tumor cells to Ki-67 and DI by flow cytometry reflect proliferative activity of STS more objectively, and together with routine histologic grading, be helpful to obtain more precise grading results in STS.

Much progress has been made in the development of successful treatment strategies, mainly conservative surgery combined with radiotherapy and chemotherapy, for patients with high-grade STS.[11] However, it is sometimes still puzzling for clinicians to decide whether individual patient may benefit from the treatment modalities or not and which one to be

chosen from varied remedies if he or she may. As the effect of adjuvant chemotherapy has not been confirmed yet, the biological behaviour of tumors needs to be judged more precisely to conduct the treatment on single patient. Until recently, we could not find any reports about results of treatment considering Ki-67 score and DI as criteria in clinical studies. Hence, future studies, based on grade, DI, and Ki-67 score, are indicated to investigate multi-modality therapy for patients with STS.

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