

Apoptotic Activities in Soft Tissue Sarcoma: Immunohistochemical Study and Their Association with Tumour Characteristics

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Abstract

Background: Many studies on the role of apoptosis in cancer development and management have been undertaken. Apoptotic activity depends partly on the balance between anti-apoptotic (Bcl-2) and pro-apoptotic (Bax) activities. This study compared Bcl-2 and Bax expression in the tumour cells and endothelial cells of tumour blood vessels in soft tissue sarcoma, and examined the association of these with tumour characteristics.

Methods: A cross sectional (retrospective) study was conducted on 101 cases of various types of soft tissue sarcoma tumour cells and endothelial cells of tumour blood vessels. The immunohistochemical expressions of Bcl-2 and Bax were compared by correlating them according to site, size, depth, tumour margin, lymph node involvement, and histological type.

Results: Higher Bax than Bcl-2 expression in tumour cells was observed, although the difference was not statistically significant. There was a significant direct association between Bcl-2 and Bax in tumour cells with endothelial cells. Among tumour characteristics, the only significant correlation was that of the Bcl-2 expression in tumour cells with tumour histological subtypes (synovial sarcoma and leiomyosarcoma).

Conclusion: The findings in this study support the role of endothelial cells in the survival and regression of tumour cells in tumour genesis. Therefore, inhibition of endothelial cell survival and activation, or induction of tumour cell apoptosis offers a promising prospect for tumour management.

Keywords: Apoptosis, Bax, Bcl-2, soft tissue sarcoma

Introduction

Soft tissue sarcomas are rare malignant tumours that encompass a histologically and anatomically diverse group of neoplasms, which share a common embryological origin from mesodermal tissue. Despite their relative rarity, there is an increased incidence and mortality, with diagnostic and therapeutic challenges due to their heterogeneity.

Development of the tumour mainly depends on a balance between cell proliferation and cell loss, by necrosis and or apoptosis (1). Since impaired apoptosis due to regulated gene mutation is one of the factors in cancer development, the induction of apoptosis to promote tumour cell death becomes one of the tumour therapeutic methods (2). Many researchers have been looking into the apoptotic activities of cancer cells recently. Apoptotic activity depends partly on the balance between

Bcl-2, which is an anti-apoptotic gene expression, and Bax, which is pro-apoptotic. This study looks at the apoptotic activities of various types of soft tissue sarcoma that can support the challenging management of this tumour, especially in the high grade type. It focuses on the immunohistochemical expression of anti-apoptotic protein (Bcl-2) and pro-apoptotic protein (Bax), in both tumour cells and the endothelial cells of blood vessels supplying the tumour of soft tissue sarcoma. Along with the expression of these proteins, we also studied their association with various tumour characteristics, namely, site, size, depth, tumour margin, lymph node involvement, and histological type. To the best of our knowledge, this is the first study to have compared the expression of Bcl-2 and Bax in tumour cells with that in the endothelial cells of blood vessels supplying the tumour.

Methodology

This was a cross sectional (retrospective) study. One hundred and one cases of various types of soft tissue sarcoma were collected from the pathology department, of the Hospital Universiti Sains Malaysia from 1999 to 2009, and their formalin-fixed paraffin-embedded tissue blocks retrieved. The inclusion criteria were based on the 2002 WHO classification of soft tissue sarcoma, including liposarcoma, malignant fibrous histiocytoma (MFH), synovial sarcoma, fibrosarcoma, rhabdomyosarcoma, leiomyosarcoma, and malignant peripheral nerve sheath tumour (MPNST). Cases with unavailable paraffin tissue blocks and very rare sarcoma such as angiosarcoma, extraskeletal ewing sarcoma, and mesenchymal chondrosarcoma were excluded from the study. Calculated by single proportion and two proportion formula, the estimated sample size was put at 103. Based on inclusion and exclusion criteria, a total of 101 cases were available.

The archived paraffin tissue blocks of soft tissue sarcoma cases were sectioned into 3–4 μm thickness and placed on poly-L-Lysine slides. After de-paraffinization, antigen retrieval was carried out with citrate buffer at pH 6.0 using a pressure cooker. Polyclonal primary antibodies were applied and incubated for 30 minutes at room temperature. Monoclonal mouse anti-human Bcl-2 (DAKO, Denmark) and monoclonal rabbit antiserum of Bax (DAKO, Denmark) were used. For a secondary antibody and detection system, HRP polymer solution, a buffered solution containing hydrogen peroxide and a detector agent of Di-amino benzidine (a chromogen in organic solvent) were used. All slides were analyzed under microscope (Olympus CX31) with 400 $\times$ magnification and scored accordingly based on the agreement scoring system.

The scoring for expression of Bcl-2 and Bax was based on combined score of qualitative and quantitative analyses. The intensity (qualitative) of the Bcl-2 and Bax immunohistochemical staining was evaluated by dividing the cytoplasmic staining reactions into four score groups: 1 = weak cytoplasmic staining intensity, 2 = moderate cytoplasmic staining intensity, 3 = strong cytoplasmic staining intensity, and 4 = very strong cytoplasmic staining intensity. The immunohistochemical staining was quantified from a total of 100 cells as follows: 0 = no positive staining, 1 = < 25% of tumour cells or endothelial

cells show cytoplasmic staining positivity, 2 = 25–50% of tumour cells or endothelial cells show cytoplasmic reactivity, 3 = 50–75% of tumour cells or endothelial cells showing cytoplasmic reactivity, and 4 = > 75% of tumour cells or endothelial cells showing cytoplasmic reactivity. A combined score for Bcl-2 and Bax immunohistochemical staining was obtained by adding the qualitative and quantitative scores; these sums were then divided into three main groups: score = 0, no immunoreactivity; score = 1–4, weak immunoreactivity; and score = 5–8, strong immunoreactivity (3). To further simplify, we condensed the results into just two categories; negative, indicating no immunoreactivity, and positive, indicating weak and strong immunoreactivity.

All the results were analyzed by using PASW Statistic 18.0 (Pearson Chi-square Test and Fisher Exact Test).

Results

Expression of Bcl-2 and Bax

Bcl-2 expression was greater in tumour cells than in endothelial cells. Among the positive cases in tumour cells 27.8% showed strong expression and 16.8% showed weak expression, as opposed to only 7.9% showing strong expression and 26% weak expression in endothelial cells. As in the case of expression of Bcl-2, the expression of Bax in tumour cells was stronger than in endothelial cells. The positive staining results were 54.5% and 48.5% in tumour and endothelial cells, respectively. The majority of the positive cases in both groups showed weak immunoreactivity. Strong positive immunostaining reactions of Bcl-2 and Bax in tumour cells and endothelial cells are shown in Figure 1a, Figure 1b and Figure 2a, Figure 2b.

Association of Bcl-2 and Bax expression between tumour cells and endothelial cells

The association between expression of Bcl-2 in tumour cells and endothelial cells is summarized in Table 1a. Only 7.9% of cases showed strong expression and about half of the cases (55.4%) indicate no reactivity in either tumour cells or endothelial cells. Using the McNemar-Bowker test, a significant association of Bcl-2 expression between the two groups was found. Table 1b shows the association between the expression of Bax in tumour cells and in endothelial cells based on category. Some 17.8% of both tumour cells and endothelial cells showed strong expression,

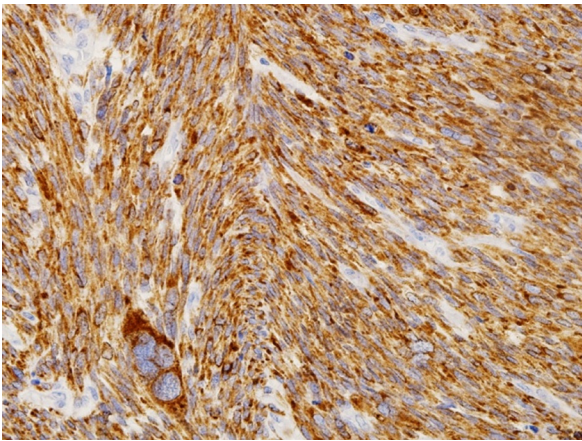


Figure 1a: Bcl-2 cytoplasmic positivity in tumour cell (400 × magnification) 175 × 130 mm (96 × 96 DPI).

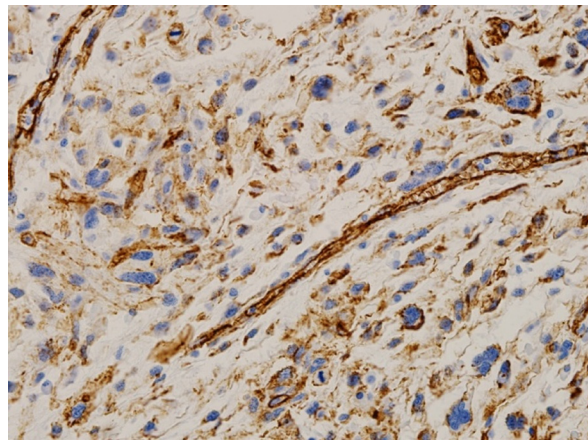


Figure 1b: Bcl-2 cytoplasmic positivity in endothelial cells (400 × magnification) 174 × 130 mm (96 × 96 DPI).

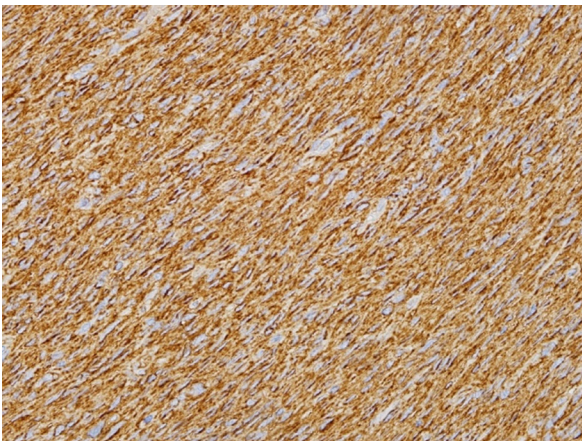


Figure 2a: Bax cytoplasmic positivity in tumour cells (400 × magnification) 175 × 131 mm (96 × 96 DPI).

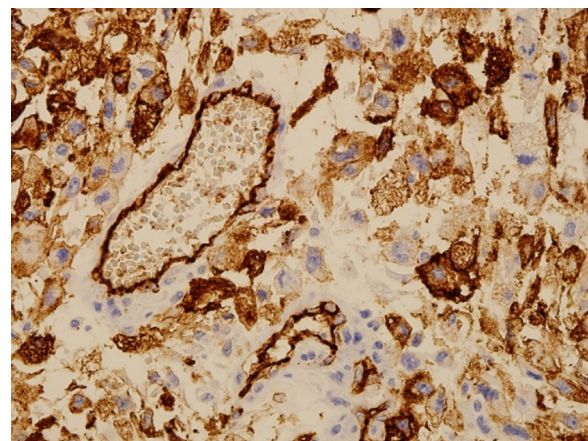


Figure 2b: Bax cytoplasmic positivity in endothelial cells (400 × magnification) 175 × 131 mm (96 × 96 DPI).

Table 1a: Association between expression of Bcl-2 in tumor cells (TC) and endothelial cells (EC) among study subjects ($n = 101$)

Variable (immunoreactivity)	Bcl-2 TC			Value ^a	Df ^b	P-value ^c
	Negative	Positive Weak	Positive Strong			
Bcl-2 EC						
No	56 (55.4%)	9 (8.9%)	2 (2.0%)	29.000	3	< 0.001
Weak	0 (0%)	8 (7.9%)	18 (17.8%)			
Strong	0 (0%)	0 (0%)	8 (7.9%)			

^aMc Nemar-Bowker Test.

^bdf = degree of freedom.

^cMc-Nemar Test.

Table 1b: Association between expression of Bax in tumor cells (TC) and endothelial cells (EC) among study subjects ($n = 101$)

Variable (immunoreactivity)	Bax-TC				Value ^a	Df ^b	P-value ^c
	Negative		Positive Weak	Positive Strong			
Bax-EC							
No	46 (45.5%)		5 (5.0%)	1 (1.0%)	14.000	3	< 0.001
Weak	0 (0%)		23 (22.8%)	8 (7.9%)			
Strong	0 (0%)		0 (0%)	18 (17.8%)			

^aMc Nemar-Bowker Test.^bdf = degree of freedom.^cMc-Nemar Test.

and 45.5% showed no expression of Bax in either. There was also a significant association of Bax expression between both groups with the McNemar-Bowker test.

Association of Bcl-2 and Bax in tumour cells with tumour characteristics

Statistical analyses were performed using the Pearson chi-square test and Fisher Exact test. The histological subtype of the soft tissue sarcoma was the only parameter to show a significant association between the expressions of Bcl-2 in tumour cells and tumour characteristics at a significant Pvalue of 0.009d (Fisher Exact Test). Among the seven types of soft tissue sarcoma included in this study (Table 2), Bcl-2 expression was very high in synovial sarcoma (100%) and leiomyosarcoma (71.4%), whereas liposarcoma, MFH, and MPNST showed less than 40% of positive cases. No significant association was noted between the Bcl-2 in tumour cells with site, size, depth, margin, or lymph node involvement using the Pearson Chi-square test. There were also no association between expression of Bax with site, size, depth, margin, lymph node involvement, or histological type of soft tissue sarcoma. Bcl-2 expression was higher in intra-abdominal soft tissue sarcoma, whereas Bax was higher in the soft tissue sarcoma of extremities. The majority of cases were larger sized tumours of more than 5 cm in diameter, of which 57.8% were negative for Bcl-2, but 57.8% positive for Bax. Regarding association with depth of tumour, both Bcl-2 and Bax showed no significant differences between superficial and deep seated soft tissue sarcoma. The majority of cases showed no lymph node involvement and their expression of both Bcl-2 and Bax showed no significant differences.

Discussion

Expression of Bcl-2 and Bax

The two most important apoptosis regulating proteins in the development and progression of malignant tumour are the functionally antagonistic Bcl-2 and Bax. In this study, the expression of Bcl-2 was seen in 45 (44.6%) cases and of Bax in 55 (54.5%) cases. Therefore, apoptotic activity is higher than anti-apoptotic in tumour cells. These finding are consistent with the findings reported by Sabah et al. (4). Unlike the expression of Bcl-2, not many studies have been done to evaluate that of Bax in soft tissue sarcoma by immunohistochemistry. In fact, only three studies have been performed to date, by Kawauchi et al., Dan'ura et al., and Sabah et al. all of which revealed Bax expression to be a common finding in soft tissue sarcoma (4–6).

Bcl-2 and Bax are expressed differently in various tissue and cells populations, and may respond differently to apoptotic stimuli (7,8). Bcl-2 is expressed mostly in the peripheral proliferating tumour areas and decreased in the areas of cell death. Contrary to Bax, it is highly expressed in the peripheral (close to stroma) and remains unchanged or even increased in the areas of cell death (9).

This study showed slightly higher expression of Bax compared to Bcl-2, as most cases were high grade (high necrotic areas). This finding supports some research which showed a strong association between Bax and Bcl-2 expression, and high-grade tumours(10–12). Although most of the cases in this study were high grade tumours, some of the cases had been treated with pre-operative chemotherapy. This might be the reason why the majority of tumours showed negative Bcl-2 (55%) and many also showed negative Bax (45%).

Table 2: Association between Bcl-2 and Bax in tumor cells (TC) with tumor characteristics

Tumour characteristics	Bcl-2 in TC		P-value ^c	Bax in TC		P-value ^c
	Negative Freq(%)	Positive Freq (%)		Negative Freq(%)	Positive Freq(%)	
Site						
Extremities	30 (52.6)	27 (47.4)	0.124	21 (36.8)	36 (63.2)	0.083
Trunk	15 (75.0)	5 (25.0)		13 (65.0)	7 (35.0)	
Intraabdominal	11 (45.8)	13 (54.2)		12 (50.0)	12 (50.0)	
Size						
< 5 cm	8 (44.4)	10 (55.6)	0.300	11 (61.1)	7 (38.9)	0.143
> 5 cm	48 (57.8)	35 (42.2)		35 (42.2)	48 (57.8)	
Depth						
Superficial	42 (56.8)	32 (43.2)	0.661	33 (44.6)	41 (55.4)	0.751
Deep	14 (51.9)	13 (48.1)		14 (48.1)	14 (51.9)	
Margin						
Negative	33 (61.1)	21 (38.9)	0.219	29 (53.7)	25 (46.3)	0.078
Positive	23 (48.9)	24 (51.1)		17 (36.2)	30 (63.8)	
Lymph node						
Negative	55 (56.1)	43 (43.9)	0.584 ^d	46 (46.9)	52 (53.1)	0.249 ^d
Positive	1 (33.3)	2 (66.7)		0 (0.0)	3 (100.0)	
Histological type						
Liposarcoma	25 (69.4)	11 (30.6)	0.009 ^d	17 (47.2)	19 (52.8)	0.250 ^d
MFH	13 (65.0)	7 (35.0)		8 (40.0)	12 (60.0)	
Synovial sarcoma	0 (0.0)	6 (100.0)		0 (0.0)	6 (100.0)	
Fibrosarcoma	4 (44.4)	5 (55.6)		4 (44.4)	5 (55.6)	
Rhabdomyosarcoma	5 (55.6)	4 (44.4)		5 (55.6)	4 (44.4)	
Leiomyosarcoma	4 (28.6)	10 (71.4)		7 (50.0)	7 (50.0)	
MPNST	5 (71.4)	2 (28.6)		5 (71.4)	2 (28.6)	

Abbreviation: Freq = Frequency.

^cPearson Chi-square Test.^dFisher Exact Test.

Association of Bcl-2 and Bax with tumour cells and in endothelial cells

This study showed that expression of both Bcl-2 and Bax is directly associated with tumour cells and endothelial cells ($P < 0.001$). A review of the literature did not show any report of an association of the expression of Bcl-2 and Bax in tumour cells with that of endothelial cells.

Apoptosis in endothelial cells may play an important role in the biology of tumour vessels, since the survival of the endothelial cells within the tumour stroma influences the survival of the tumour cells themselves. Endothelial cells isolated from tumour vessels appear to be more resistant to apoptosis than endothelial cells isolated from

vessels of corresponding normal tissues (13). This supports our finding of a direct association between the expression of Bcl-2 in tumour cells and in endothelial cells. Based on these results, the inhibition of endothelial survival factors or the activation of endothelial cell death presents as an attractive anti-tumour strategy, especially when used to supplement strategies directed at the tumour cells themselves (14,15). Several anti-angiogenic agents in clinical trials for cancer treatment appear to exert at least part of their anti-tumour action by promoting endothelial cells apoptosis.

The apoptosis in endothelial cells may be the triggering factor that selectively causes

an increased apoptosis in tumour cells. This might be the reason for our findings that the higher expression of Bax indicates a heightened apoptotic activity. However our results may not reflect the actual apoptotic process in which multiple proteins are known to interact with Bcl-2 and Bax, in that the apoptotic rate of a tumour in experimental models is not only influenced by absolute Bcl-2 or Bax levels, but also by the ratio of Bcl-2 to Bax (16).

Association of Bcl-2 with Bax in tumour cells with tumour characteristics

The only significant association in our study was that of the expression of Bcl-2 in tumour cells with histological subtypes. In this study, the expression of Bcl-2 and Bax were 100% in synovial sarcoma. Bcl-2 expression was also commonly seen in leiomyosarcoma (71.4%) and fibrosarcoma (55.6%), whereas Bax expression was seen in MFH (60%), fibrosarcoma (55.6%), liposarcoma (52.8%) and leiomyosarcoma (50.0%). Rhabdomyosarcoma and MPNST were expressed in less than 50% of both Bcl-2 and Bax proteins. These findings were consistent with the observation reported by Sabah et al. and Hasegawa et al., which revealed the significant association between the Bcl-2 expression and histological subtypes (4,7).

About 80–100% of synovial sarcoma has been reported to exhibit Bcl-2 immunoreactivity (18,19). The possibility of an association between the Bcl-2 expression in synovial sarcoma and the characteristic chromosomal translocation t(X;18) has been proposed (19).

In contrast to this study, Sabah et al. and Hasegawa et al. have revealed that Bcl-2 expression was not a common finding in other soft tissue sarcoma, such as leiomyosarcoma, MFH and fibrosarcoma (4,17). In view of the diversity of Bcl-2 expression among the various histological subtypes of soft tissue sarcoma, Hasegawa et al. suggested that Bcl-2 can be used together with CD34 in the diagnosis of solitary fibrous tumour to distinguish this entity from other spindle cell neoplasms, including monophasic fibrous synovial sarcoma (Bcl-2 positive, but CD34 negative) and other tumours, such as leiomyosarcoma, MFH and fibrosarcoma, which are frequently Bcl-2 negative (17).

This study noted no significant association between the expression of Bcl-2 and Bax and tumour site, size depth, margin, and lymph node involvement. There was also no association

between the expressions of Bax with histological subtypes. In contrast, Nakanishi H. et al. showed the expression of Bcl-2 to be correlated with a larger tumour size. Thus, it is a useful marker for predicting prognosis (20).

Only a few immunohistochemical studies have been done on Bax expression in soft tissue sarcoma, none of which showed any relationship between Bcl-2 and Bax expression and tumour grade (4,6).

Conclusion

This study analyzed the apoptotic activities (Bcl-2 and Bax) of soft tissue sarcoma in both tumour cells and endothelial cells of tumour blood vessels, and their association with tumour characteristics. It was found that apoptotic activity was more prominent in tumour cells than in the endothelial cells; pro-apoptotic activity of tumour cells is directly associated with that of endothelial cells ($P < 0.001$); anti-apoptotic activity of tumour cells is directly associated with that of the endothelial cells ($P < 0.001$); and anti-apoptotic activity of tumour cells is directly associated with histological subtypes of the soft tissue sarcoma ($P < 0.001$).

In conclusion, apoptotic activity plays an important role in the development and progression of soft tissue sarcoma. The findings of this study support the role of endothelial cells in the survival and regression of tumour cells in tumour genesis. Therefore, the inhibition of endothelial cell survival and activation or induction of tumour cell apoptosis offers a promising prospect for tumour management.

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Authors' contributions

Conception and design: TTW, HJ, YY
Analysis and interpretation of the data: YY
Drafting of article: TTW, YY
Final approval of article: TTW

Conflict of interest

Nil.

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