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Cell-type- and tumour-type-related patterns of bcl-2 reactivity in mesenchymal cells and soft tissue tumours

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Abstract Bcl-2 is one of the many proteins that regulate programmed cell death and is overexpressed in B-cell lymphomas. The expression of bcl-2 in mesenchymal cells and soft tissue tumours was the subject of this study. Normal mesenchymal tissue and representative cases of soft tissue tumours of different types (n>200) were investigated immunohistochemically for bcl-2 expression. Although bcl-2 expression was normally relatively restricted to some smooth muscle cells and neural cells, bcl-2 immunoreactivity was widespread in different types of soft tissue neoplasms, both benign and malignant. Consistently positive tumours included solitary fibrous tumour, haemangiopericytoma, schwannoma and synovial sarcoma. The few soft tissue tumours that were consistently negative for bcl-2 included nodular fasciitis and desmoid tumour. Leiomyomas and leiomyosarcomas were heterogeneous; all uterine leiomyomas were bcl-2 positive, but all oesophageal leiomyomas were negative, paralleling the reactivity observed in the smooth muscle at those sites. Gastrointestinal stromal tumours showed bcl-2 reactivity; this was less consistent in malignant tumours. Along the malignancy gradient, there was no consistent trend in the bcl-2 reactivity. Dermatofibrosarcomas showed increase of bcl-2 expression with fibrosarcomatous transformation, whereas smooth muscle sarcomas and malignant peripheral nerve sheath sarcomas were less consistently positive than the corresponding benign neoplasms. We conclude that bcl-2 expression is widespread in soft tissue tumours, but shows constitutional expression patterns that are often parallel to the normal tissue counterparts. Compared with benign soft tissue tumours, bcl-2 expression is often reduced in sarcomas, but it cannot be used as a prognostic marker without correlation of the data to its phenotypic expression patterns.

Key words Bcl-2 · Soft tissue tumours

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Introduction

Bcl-2 oncogene and its encoded protein were originally discovered in follicular B-cell lymphomas. The *bcl-2* gene is overexpressed, on the basis of a t(14;18) translocation that activates bcl-2 through the juxtaposition of promoter elements of the immunoglobulin heavy chain gene and the *bcl-2* gene [4, 24]. Although initially described in follicular lymphomas, bcl-2 is known to be widely expressed in different lymphomas independently of the t(14;18) translocation [16, 17]. Bcl-2 is also expressed in many non-neoplastic lymphoid cells, specifically including those B-cells that have been challenged by antigen and selected for prolonged survival [6, 17, 19], certain non-lymphoid cells, especially basal cells and other proliferating epithelial cells, many neural cells, and myometrium [8, 11]. A differential expression pattern of bcl-2 exists in fetal and adult tissues, especially at stromo-epithelial junctions, suggesting a role in morphogenesis [10]. In vitro observations suggest that bcl-2 has a role in neuronal differentiation [27].

Functionally bcl-2 protein differs from other oncogene products in that it does not promote cellular proliferation, but instead inhibits apoptotic cell death [2, 6, 20]. Bcl-2 is an inner membrane protein present in mitochondria and rough endoplasmic reticulum, but its mechanism of action is not well understood [7, 26].

Because *bcl-2* oncogene promotes cellular survival, correlations with its expression patterns and clinical behaviour have been examined in many types of tumours. In breast carcinomas, bcl-2 has been observed in better differentiated carcinomas (in parallel with bcl-2 expression in normal epithelia), but it is often absent in poorly differentiated tumours, including hormone receptor-negative cases [1]. In thyroid carcinomas, bcl-2 expression also correlates with a higher tumour differentiation level [12]. In one study, those non-small-cell lung carcinomas that expressed bcl-2 were shown to have a better prognosis than the bcl-2-negative cases [18]. However, in small round cell tumours, such as neuroblastoma [3] and Ewing's sarcoma and related tumours [23], bcl-2 expression correlated with unfavourable histology and a less favourable clinical outcome.

Bcl-2 has also been found in some soft tissue tumours, notably tumours of muscle cell origin [22], and solitary fibrous tumours [5]. In soft tissue sarcomas, bcl-2 expression has been suggested as a favourable prognostic marker [14]. However, the distribution of bcl-2 has not been systematically studied in soft tissue tumours.

The functional interpretation of bcl-2 expression is complex considering that it acts together with a number of other proteins, including bcl-2 homologues such as mcl-1, bcl-x, and bax, which has the opposite effect, i.e. promotion of apoptosis [6, 9, 15]. Other proteins related to apoptosis regulation include p53, c/myx and fas/APO-I; the last-mentioned acts through receptor-mediated apoptosis [2].

Materials and methods

A representative selection of soft tissue tumours with emphasis on spindle cell neoplasms was obtained from the files of the authors and from the soft tissue registry of the Armed Forces Institute of Pathology (a total of 213 cases). These tumours had been extensively characterized immunohistochemically to establish the diagnosis. Selected normal tissues (5–10 examples of each tissue and cell type) were also studied. Some of these tissues were adjacent to tumour, but non-neoplastic tissue specimes were also studied.

The tumours were evaluated immunohistochemically for bcl-2 oncoprotein using monoclonal antibody (Clone 124, Dako, Carpinteria, Calif., dilution 1:40). Immunohistochemistry was performed via avidin–biotin complex peroxidase (ABC) detection and amplification system (Vector Elite, Vector Labs, Burlingame, Calif.). The colour was developed with diaminobenizidine. Immunostaining was preceded by a microwave energy-based epitope retrieval using a commercial heat-induced epitope retrieval buffer (Tech-Mate HIER- buffer). The primary antibody was incubated overnight at room temperature, followed by completion of the detection, colour development and counterstaining (haematoxylin) next day using the Techmate 1000 automatic immunostainer (Ventana medical systems, Tucson, Ariz.). The adequacy of epitope retrieval was controlled by using a positive control (normal tonsil) showing bcl-2 reactivity in the interfollicular lymphoid tissue and scattered intrafollicular lymphocytes. In addition, bcl-2-positive lymphocytes were sought in each negative case and those cases that did not show bcl-2 reactivity in any normal lymphocytes were eliminated. A total of 8 cases (3.8%) were eliminated, representing specimen-related epitope retrieval failure. Positive cases were evaluated semiquantitatively for the proportion of positive cells.

Results

Bcl-2 immunoreactivity was successfully demonstrated in 205/213 (96.2%) of the specimens evaluated, as judged either by verification of bcl-2-positive lymphocytes in the tumour or by the presence of specific immunostaining in the tumour cells.

In normal mesenchymal tissues, bcl-2 was present in peripheral nerves and in the autonomous nerve trunks in the intestinal wall. Smooth muscle cells of erector pili and intestinal walls were negative, whereas myometrium was variably positive. Vascular smooth muscle cells showed variable bcl-2 reactivity in different areas. Skeletal muscle was generally negative, but atrophic or regenerative muscle fibres adjacent to tumours were often positive. Dermal and other connective tissue fibroblasts and vascular endothelial cells were negative.

The patterns of bcl-2 reactivity in soft tissue tumours are summarized in Table 1. In general, bcl-2 reactivity was widespread in both benign and malignant soft tissue tumours of different cell lineages, but the reactivity was often less uniform in malignant tumours.

In two subsets of fibroblastic tumours, bcl-2 reactivity was consistently absent. Nodular fasciitis and desmoid fibromatosis showed bcl-2 reactivity only in scattered small lymphocytes, while the lesional cells were negative (Fig. 1).

Typically strong and uniform bcl-2 reactivity was present in solitary fibrous tumour (Fig. 2), haemangiopericytoma, and spindle cell lipoma. In dermatofibrosarcoma protuberans bcl-2-positive cells were seen focally in a minority of cases, but most cases of DFSP with fascicular pattern and increased cellularity (fibrosarcomatous transformation) showed bcl-2-positive cells, although usually only focally, often perivascularly.

Among smooth muscle tumours and gastrointestinal stromal tumours (GISTs, formerly called cellular leiomyomas), the patterns of bcl-2 reactivity varied. While all uterine leiomyomas were bcl-2 positive (Fig. 3), oesophageal leiomyomas were entirely bcl-2 negative (Fig. 4). Benign gastrointestinal stromal tumours, which were typically negative for desmin and positive for CD34, were uniformly bcl-2 positive (Fig. 5). Similar, strong bcl-2 reactivity was seen in stromal tumours of both spindle cell and epithelioid features ('leiomyoblastomas'). However, malignant GISTs showed less uniform reactivity for bcl-2, often with a heteregeneous mosaic-like pattern.

Leiomyosarcomas of uterus showed variable bcl-2 reactivity in most cases (6/9), and a mosaic-like pattern with alternating positive and negative areas was seen in about half of the cases (Fig. 6). In contrast, leiomyosarcomas of peripheral soft tissues were less commonly and only focally bcl-2 positive (3/6).

Benign schwannomas showed bcl-2 reactivity in the cellular schwannian components, whereas the loose, myxoid ("Antoni-B") areas were negative. In neurofibromas, a less dense population of bcl-2-positive cells was observed. In malignant peripheral nerve sheath sarco-

Table 1 Summary of the patterns of Bcl-2 expression in soft tissue tumours.

Tumour type	Positive/ all cases	Description of patterns of reactivity
Desmoid tumour	0/10	Always negative
Nodular fasciitis	0/5	Always negative
Solitary fibrous tumour	22/22	Strong reactivity in 20/22 cases, focal reactivity in 2 cases
Dermatofibrosarcoma protuberans (DFSP)	2/10	Positive cases showed focal, limited reactivity
DFSP, with fibrosarcomatous transformation	9/10	Reactivity focal, typically perivascular
Malignant fibrous histiocytoma, storiform-pleomorphic	6/11	Three cases extensively positive, three focally positive
Spindle cell lipoma	5/5	Spindle cells positive in all cases
Leiomyoma, uterine	9/9	Most cells positive in all cases
Leiomyoma, oesophageal	0/7	Always negative
Gastrointestinal stromal tumour (GIST) benign	12/12	Most tumour cells positive
GIST, malignant	9/9	5/9 widely positive, 4/9 focally positive
Leiomyosarcoma, uterine	9/11	Most cells positive in 7/9 of the positive cases, 2 cases focally positive
Leiomyosarcoma, peripheral	3/6	Focal, limited reactivity in the three positive cases
Hemangioperiocytoma	19/21	Strong reactivity in all cells in positive cases
Schwannoma, soft tissue	12/12	Widespread reactivity in most cases, one focally +
Malignant peripheral nerve sheath tumour (MPNST)	8/10 4/10	Marked intratumoural heterogeneity in the positive cases, cases widely positive
Synovial sarcoma, biphasic glandular component	3/5	Variable reactivity. Entirely negative in 2/5 cases, heterogeneously positive in 3/5 cases
Synovial sarcoma, spindle cell ("monophasic")	24/24	Widespread reactivity in all cases, most cells positive
Synovial sarcoma, poorly differentiated	6/6	Most cases show widespread reactivity
Total	205	

mas, bcl-2 reactivity was observed in most cases, but typically there was intratumour heterogeneity with a patchy pattern of immunoreactivity.

Synovial sarcomas displayed distinctively different patterns of bcl-2 expression in different areas. While the stromal components of spindle cell tumours and the monophasic and poorly differentiated spindle cell tumours were bcl-2 positive, the epithelial cells were mostly negative, contrasting with the staining of the spindle cell elements (Fig. 7). However, in two cases the opposite pattern was seen, with bcl-2-positive epithelial cells and negative stromal cells. The poorly differentiated synovial sarcomas with haemangiopericytoma-like pattern were also bcl-2 positive.

Discussion

This study has yielded baseline information for prognostic studies and for the evaluation of bcl-2 expression as a differential diagnostic variable. However, we are cautious about a functional interpretation of bcl-2 expression, since the action of bcl-2 and those of a complex group of other apoptosis regulators, including the homologous proteins of the bcl-2 family, mcl-1, bax, and bcl-x, some of which have opposite effects to bcl-2, in that they promote apoptosis [2, 6], are interdependent. Furthermore, other regulator proteins are involved and include p53, c-myc and the receptor-mediated apoptosis by the Fas/APO-I (CD95) system [2, 6].

Bcl-2 protein shows characteristic, complex expression patterns in mesenchymal tissues that do not always correlate with the cell lineage, but sometimes vary within cells of the same lineage between different sites. Understanding of the patterns of bcl-2 distribution in normal tissues is important for understanding its distribution in tumours. Compared with normal tissues, many, but not all, mesenchymal tumours show increased bcl-2 expression. This observation is in parallel with the increased bcl-2 reactivity in fetal vs adult tissues [10] and indicates that tumours may show "retrodifferentiation" and phenotypically resemble fetal more than adult tissues.

Bcl-2 reactivity in found in peripheral nerves, the intestinal autonomous nerve system, and some smooth muscle cells (especially myometrium) and some vascular smooth muscle cells. It has been noted earlier that bcl-2 is present in many hormonally regulated cells, such as luminal epithelial cells of breast and hormone-receptor-positive breast carcinomas [1, 11]. However, the marked difference between smooth muscle of different locations has not been explored previously. While the myometrium is bcl-2 positive, the erector pili muscles of skin and gastrointestinal smooth muscle are remarkably negative. Our findings are in agreement with previous investigators who found bcl-2 reactivity in peripheral nerves, ganglion cells and the myometrium [8, 11].

Bcl-2 expression in fibrous soft tissue tumours is variable, but some tumour-type-related expression patterns can be observed. While many fibroblastic tumours, such as solitary fibrous tumour and a haemangiopericytoma,

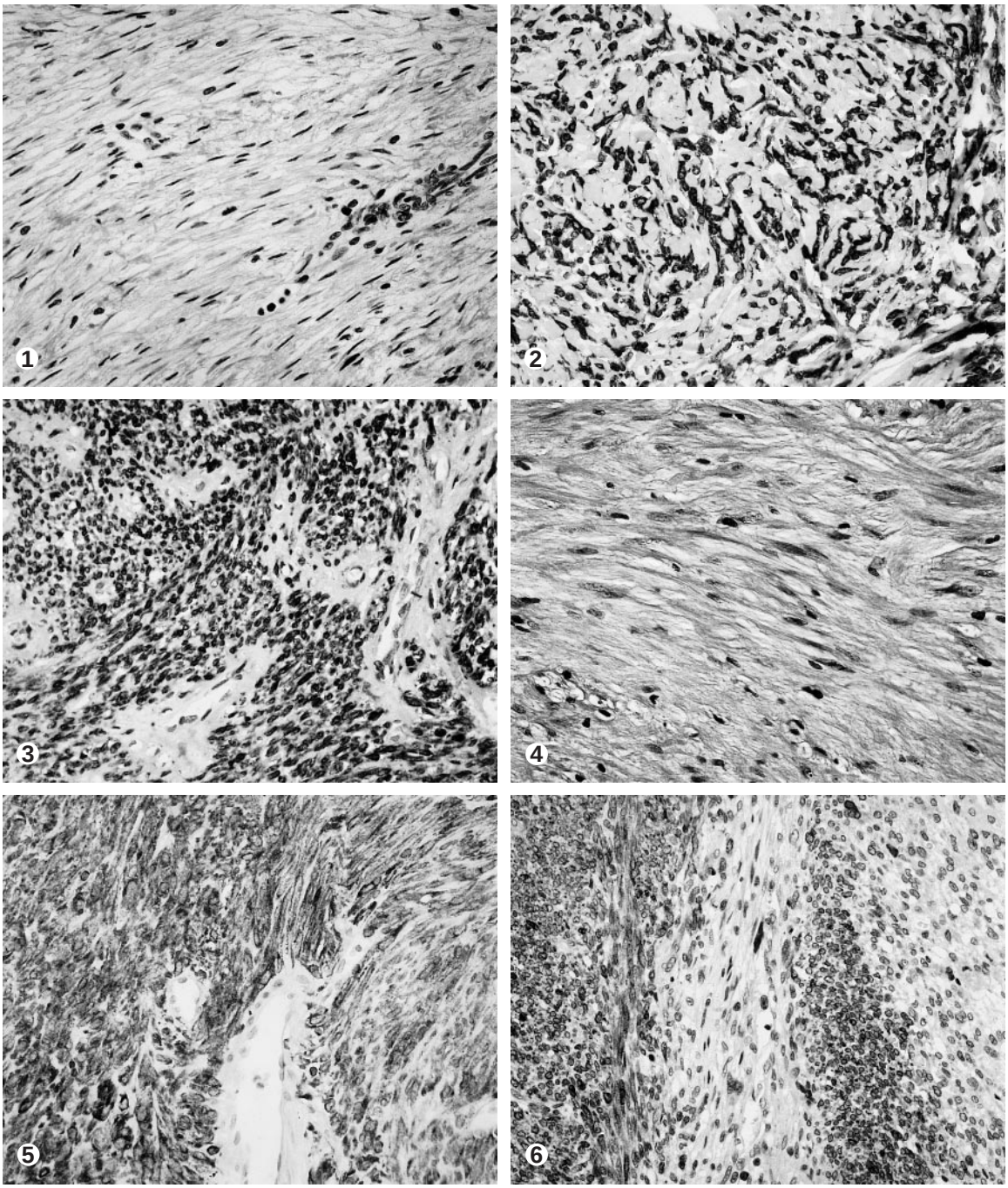


Fig. 1 Desmoid tumour cells are negative for bcl-2. Note scattered positive lymphocytes around the vessel. ABC-immunoperoxidase with haematoxylin counterstain, ×240

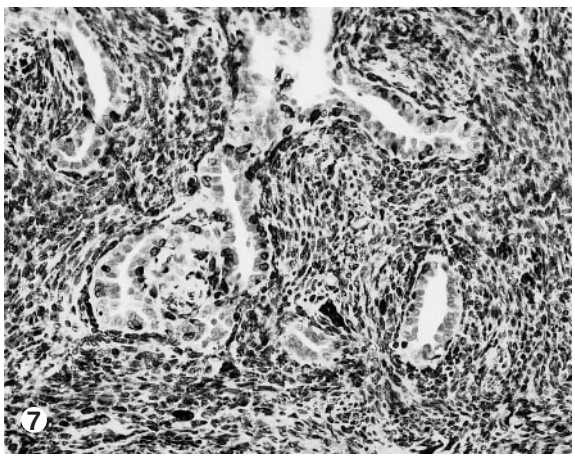
Fig. 2 Solitary fibrous tumour is strongly bcl-2 positive. ABC-immunoperoxidase with haematoxylin counterstain, ×240

Fig. 3 Uterine leiomyoma is strongly bcl-2 positive. ABC-immunoperoxidase with haematoxylin counterstain, ×240

Fig. 4 Oesophageal leiomyoma is bcl-2 negative. ABC-immunoperoxidase with haematoxylin counterstain, ×240

Fig. 5 A gastric stromal tumour of spindle cell type is strongly positive for bcl-2. ABC-immunoperoxidase with haematoxylin counterstain, ×240

Fig. 6 Uterine leiomyosarcoma shows bcl-2-positive and -negative areas in a mosaic-like pattern. ABC-immunoperoxidase with haematoxylin counterstain, ×240



are strongly positive, some are consistently negative, including desmoid tumour and nodular fasciitis. In nodular fasciitis, lack of bcl-2 expression correlates with the limited life of the nodular fasciitis cells, while a similar lack of bcl-2 was observed in desmoid tumours, although these tumours have the capacity to recur relentlessly. Tumour behaviour cannot be predicted from bcl-2 expression status alone.

Among smooth muscle tumours different patterns of bcl-2 expression were noted. While all uterine leiomyomas were bcl-2 positive, all oesophageal leiomyomas were negative. This finding seems to correlate with the expression patterns of bcl-2 in the corresponding non-neoplastic smooth muscle. Gastrointestinal stromal tumours (GISTs), previously often referred to as cellular leiomyomas, were bcl-2 positive and showed a marked contrast with oesophageal leiomyomas. This illustrates the different phenotypic properties between true leiomyomas and GISTs; the latter are known to be generally negative for muscle cell markers but positive for CD34, in contrast with true leiomyomas, which are CD34 negative [13, 25].

Malignant mesenchymal neoplasms sometimes showed a weaker bcl-2 expression than to benign tumours. Uterine leiomyosarcomas showed heterogeneous bcl-2 expression, while uterine leiomyomas were always positive. Similarly, malignant gastrointestinal stromal tumours showed a less consistent, often patchy bcl-2 expression compared with their benign counterparts. In a similar way, malignant nerve sheath tumours expressed less bcl-2 than benign ones [21]. One study suggested that loss of bcl-2 correlated with a poorer prognosis in soft tissue tumours [14]. However, the study material was relatively small, and no correlation was made with the phenotypic patterns of bcl-2 expression. It seems likely that the partial, variable loss of bcl-2 expression in malignant tumours accounts for the unfavourable prognosis of bcl-2-negative sarcomas.

However, some low-grade sarcomas, such as dermatofibrosarcoma protuberans, appear to lack bcl-2 expression. Nonetheless, these tumours are known to have an excellent prognosis if treated with adequate surgery. This illustrates that tumour behaviour cannot be assessed on the sole basis of bcl-2 expression and that the constitutional patterns of bcl-2 expression should be considered in all studies correlating bcl-2 expression and tumour behaviour.

Biphasic synovial sarcoma usually showed strong bcl-2 reactivity in the spindle cell component but limited, if any, reactivity in the epithelial component. Such a differential pattern of bcl-2 reactivity has previously been observed in fetal kidney, where the mesenchymal cells that give rise to tubules are bcl-2 positive, while the tubules are negative. In synovial sarcoma, the bcl-2-positive mesenchymal cells may represent a similar stem cell pool, as has been suggested for the bcl-2-positive mesenchymal condensations that give rise to tubules in fetal kidney [10]. Bcl-2 may have a similar role in the morphogenesis of biphasic soft tissue tumours to that suggested for it in fetal tissues.

We have evaluated the patterns of bcl-2 expression in mesenchymal tissues and soft tissue tumours, and found cell-type and site-specific patterns with a tendency to reduced bcl-2 expression in some but not all malignant tumours. However, the bcl-2 expression in soft tissue tumours cannot be correlated uniformly with malignancy and prognosis. Rather, the patterns of bcl-2 expression may differ within a histogenetic group (for example among smooth muscle tumours). Patterns of bcl-2 expression should be considered when the possible prognostic significance of bcl-2 expression is evaluated.

◀ **Fig. 7** Synovial sarcoma shows blc-2 reactivity in the spindle cell component and in a a minority of glandular epithelial cells, while most epithelial cells are negative. ABC-immunoperoxidase with haematoxylin counterstain, ×240

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