Study of the immunoexpression of Bcl-2 by a cutaneous granular cell tumor

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ABSTRACT

Background: Cutaneous granular cell tumors have only rarely been examined for Bcl-2, a marker that is expressed by granular cell tumors from other parts of the body.

Objective: We retrospectively studied three cases of cutaneous granular tumors from our archives.

Methods: We immunohistochemically tested for neuron-specific enolase (NSE), CD68 (KP1), Bcl-2, and S-100. In Cases 1 and 3, we also tested for CD34, HMB-45, and melan-A. In Case 3, we additionally tested for smooth muscle actin, CD68-PGM1, cytokeratin AE1-AE3, epithelial membrane antigen, desmin, CD1a, and CD117.

Results: None of our cases presented any of the histological markers traditionally considered to be indicators of potential malignancy in granular cell tumors. All cases strongly expressed S-100, CD68, NSE, and Bcl-2. Case 3 also expressed CD68-PGM1. The rest of the markers were not expressed by the tumors.

Conclusions: Bcl-2 is expressed by cutaneous granular cell tumors.

KEY WORDS

granular cell tumor, Bcl-2, apoptosis, schwannoma

Background and objective

Granular cell tumors (GCTs) of the skin are uncommon (1). Therefore, dermatopathology has had few opportunities to study certain antibodies present in these tumors. That is the case for Bcl-2, a marker expressed by the GCTs of many organs (2–5). Cutaneous GCTs have rarely been examined for Bcl-2. Vera-Sempere's (6) study was one such case where they were. In this report, we examine the immunoexpression of Bcl-2 in three cases of GCTs of the skin and address some of the questions that our results raise regarding the marker and this type of tumor.

Methods

We retrospectively studied three cases of cutaneous granular tumors from our archives. The slides of the three cases and clinical histories of the patients were reviewed. The following immunohistochemical

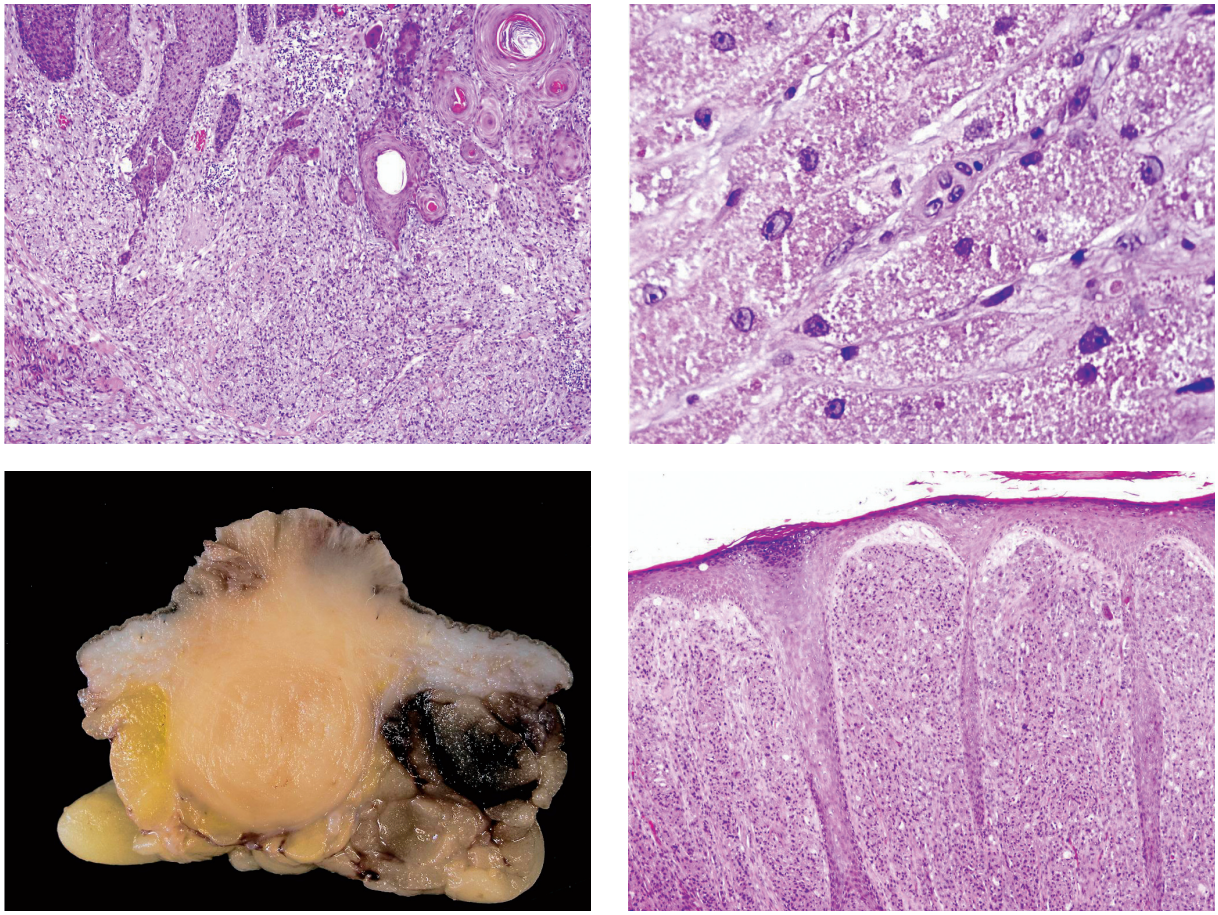

Figure 1. Case 1 (top left) showed an intense epidermal hyperplasia, mimicking a squamous cell carcinoma. The cellular morphology was bland in all cases, without atypical signs as shown in the top right photo, which corresponds to Case 2. Photos from Case 3 are shown at the bottom, showing similar morphologic features to the other cases.

panel was evaluated for each of the biopsies: neuron-specific enolase (NSE) (*Dako, monoclonal mouse anti-human, clone BBS/NC/VI-H14, isotype IgG1, kappa, code M0873*), Bcl-2 (*Dako, monoclonal mouse anti-human, clone 124, isotype IgG1, kappa, code M0887*), CD68 (*Dako, monoclonal mouse anti-human, clone KP1, isotype IgG1, kappa, code M0814*), and S-100 (*Dako, polyclonal rabbit anti-S100, code Z0311*). CD34 (*Dako, monoclonal mouse anti-human CD34 class II, clone QBEnd 10, isotype IgG1, kappa, code M7165*), HMB-45 (*Dako, monoclonal mouse anti-human melanosome, clone HMB-45, isotype IgG1, kappa, code M0634*), and melan-A (*Dako, monoclonal mouse anti-human, clone A103, isotype IgG1, kappa, code M7196*) were also tested for in Cases 1 and 3.

In Case 3, we additionally tested for smooth muscle actin (SMA) (*Dako, monoclonal mouse anti-human, clone 1A4, isotype IgG2a, kappa, code M0851*), CD68-PGM1 (*Dako, monoclonal mouse anti-human, clone PG-M1, isotype IgG3, kappa, code M0876*), cytokeratin AE1-AE3 (*Dako, monoclonal mouse anti-human, clone AE1/AE3, isotype IgG1, kappa, code M3515*), epithelial membrane antigen (EMA) (*Dako, monoclonal mouse anti-human, clone E29, isotype IgG2a, kappa, code M0613*), desmin (*Dako, monoclonal mouse anti-human, clone D33, isotype IgG1, kappa, code M0760*), CD1a (*Dako, monoclonal mouse anti-human, clone O10, isotype IgG1, kappa, code M3571*), and CD117 (*Dako, polyclonal rabbit anti-human, c-kit, code A4502*).

Results

The diagnosis was corroborated in all three cases. Table 1 shows the clinical details. In Case 1, an intense epidermal hyperplasia was seen overlying a GCT with a maximum diameter of 2 cm (Fig. 1, top left). The morphology of the tumor was that of a classic GCT, with thick bundles of tumor cells infiltrating the reticular and papillary dermis. The cells had an abundant granular cytoplasm, having central nuclei without atypias. The nucleoli were small or non-evidenced. No areas of necrosis were evidenced.

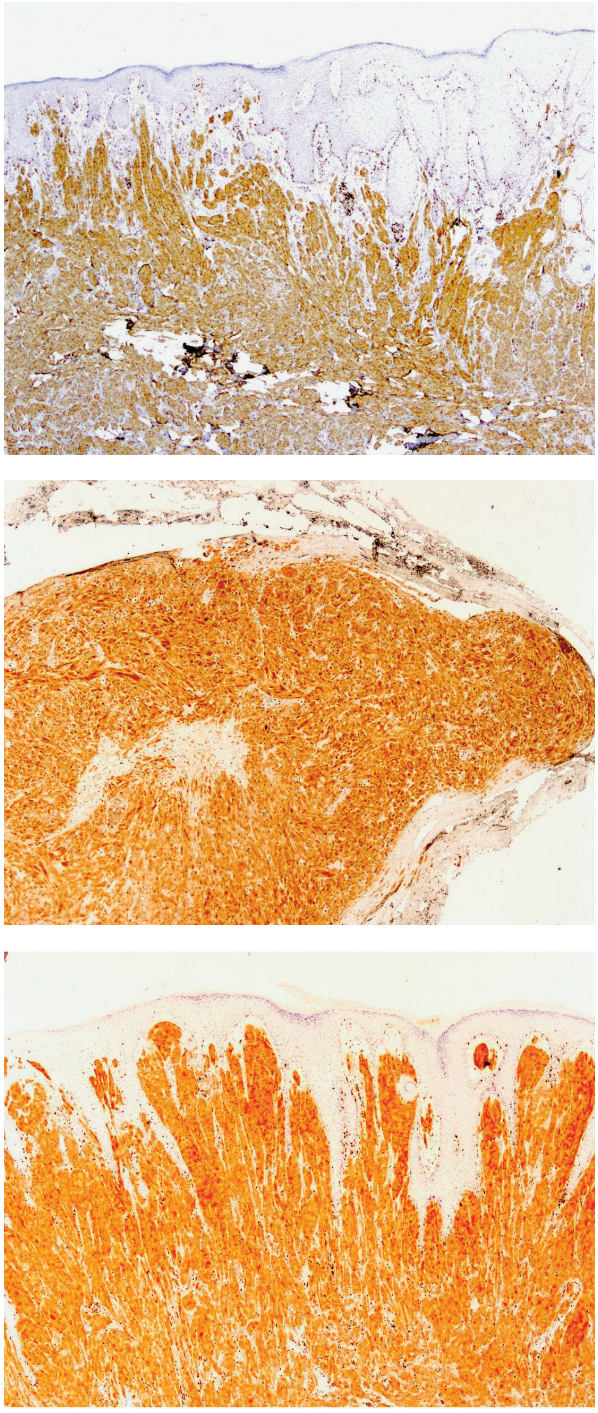

Figure 2. Intense Bcl-2 immunoexpression by the three cases (1: top, 2: middle, 3: bottom).

In Case 2, a dermal and hypodermal tumor 0.8 cm in diameter with moderate hyperplasia of the overlying epidermis was seen. The cells consisted of infiltrating bundles with no atypical features. No mitoses were found in 10 high power fields (HPFs). The cytoplasms were abundant and granular, and the nuclei were regular, with small nucleoli (Fig. 1, top right).

In Case 3, a yellow tumor 1.9 cm in diameter was evidenced in the dermis and epidermis (Fig. 1, bottom left). The tumor did not contact the overlying epidermis, which appeared to be hyperplastic (Fig. 1, bottom right). The tumor consisted of compact nests of cells, which had a large granular cytoplasm. No mitoses were evidenced in 10 HPFs. There were no areas of necrosis. Nucleoli were easily evidenced in many cells, but the chromatin was fine and granular.

None of the cases showed any of the following morphologic features: necrosis, spindling of the tumor cells, vesicular nuclei with large nucleoli, increased mitotic rate, a high nucleocytoplasmic ratio, or pleomorphism. All cases showed an intense expression of S-100, CD68, NSE, and Bcl-2 (Fig. 2). Information regarding the other immunohistochemical markers is shown in Table 1. There were no metastases or recurrences during follow-up periods of 10 months (Case 1), 12 years (Case 2), and 2 months (Case 3).

Discussion

Cutaneous GCTs are infrequently discussed in the literature. Torrijos-Aguilar et al. reported finding 13 cases of GCTs of the skin in the archives of a university hospital over a period of 27 years (1). This type of tumor usually expresses S-100, peripheral nerve myelin proteins, PGP 9.5, vimentin, NSE, CD68, and NKI-C3 (7–9).

Bcl-2 expression is seen in lymphomas, leukemias, and in certain carcinomas: prostate (10), lung (11), breast (12), pancreas (13), and gallbladder (14). It is also seen in mesotheliomas (15), salivary gland tumors (16), synovial sarcomas (17), and in tumors of muscle origin such as leiomyomas, leiomyosarcomas, or rhabdomyosarcomas (18). Bcl-2 is also occasionally expressed by liposarcomas (18) and by the ambiguous cellular category of malignant fibrous histiocytomas (18). It has also been demonstrated that solitary fibrous tumors express Bcl-2 (19, 20). Other tumors that are known to express Bcl-2 are hemangiopericytomas and schwannomas (21).

Suster et al. published an exhaustive study of Bcl-2 expression using several soft tissue tumors (22). Bcl-2 was expressed in all of their cases of spindle cell lipomas, "dedifferentiated" liposarcomas, fibrosarcomas, dendritic fibromyxolipomas, Kaposi's sarcomas, solitary fibrous tumors, gastrointestinal stromal tumors, synovial sarcomas, and fibrosarcomas (22). None of their cases of nodular fasciitis, fibromatosis, dermatofibroma, leiomyoma, and leiomyosarcoma expressed the marker (22). Most of their cases of dermatofibrosarcoma protuberans, low-grade myxofibrosarcomas, and schwannomas also tested positive. The storiform/pleomorphic sarcomas and gastrointestinal sarcomas

in their study showed variable expression, and the neurofibromas showed weak expression (22).

In GCTs from regions of the body other than the skin, Bcl-2 has been investigated in some cases and showed immunoexpression in tumors of the breast (4), stomach (3), and bronchi (5), and in oral granuloma cell tumors (2). Laryngeal GCTs have shown immunoexpression of Bcl-2 only in occasional cells (23).

In dermatopathology, some cutaneous tumors with "granular" changes such as granular basal cell carcinomas (24) express this marker, but it should be remembered that common basal cell carcinomas also do (25). Occasionally, Bcl-2 has been reported in cutaneous GCTs. Vera-Sempere et al. published two cases of GCTs of the breast skin and had positive results for Bcl-2 from an immunohistochemical report (6). The current report supports evidence for the expression of Bcl-2 by cutaneous granular tumors, similar to GCTs in other regions of the body. Our findings raise the following questions:

Does Bcl-2 expression by cutaneous GCTs explain their nature?

GCTs were first described by Weber in 1854 (26). In 1926, Abrikossoff reported a GCT of the tongue and considered it to have derived from striated muscle (27). This origin was highly debated in the literature, although it is now mostly accepted that these tumors are derived from Schwann cells, according to immunohistochemical and ultrastructural studies (28–30). Bcl-2 is expressed by normal neural cells and their related neoplasms (21). In particular, the positive expression of the marker in schwannomas has been reported (31, 32). However, Bcl-2 is not usually expressed by normal striated muscle (2, 21), which would support the schwannomatous nature of GCTs and, from our report, also of cutaneous GCTs. It is worth mentioning also that some studies have shown immunoexpression of the marker by rhabdomyosarcomas (18).

Can Bcl-2 expression by cutaneous GCTs be diagnostically helpful?

We believe that the immunoexpression of Bcl-2 by cutaneous GCTs is more useful for academic research than as a diagnostic tool. This type of tumor rarely presents diagnostic difficulty, and the main differentials are rhabdomyomas and histiocytic reactions. Although rhabdomyomas (non-cutaneous) have failed to show Bcl-2 expression (33), histiocytes do show the marker under certain circumstances (34).

Does Bcl-2 immunoexpression have any prognostic significance?

Bcl-2 immunoexpression has a prognostic significance in other cutaneous tumors such as Kaposi's sarcomas (35, 36). The marker has been studied in cases of malignant fibrous histiocytomas and correlated with recurrence and metastases (37). Nevertheless, the expression of Bcl-2 does not imply "malignancy" per se, and it has not been correlated with a bad prognosis in all tumors. Many malignant mesenchymal neoplasias exhibit a weaker expression of the marker than do their benign counterparts (21). There is an inverse correlation between Bcl-2 expression and prognosis in ovarian cancer, prostate cancer, non-small cell lung cancer, follicular thyroid cancer, neuroblastoma, and breast cancer (37).

Cruz-Mojarrieta et al. found immunoexpression of Bcl-2 in two cases of malignant GCTs in soft tissue (38). Fanburg-Smith et al. studied Bcl-2 immunoexpression in malignant and benign GCTs of soft tissues and concluded that "Bcl-2 values were not statistically significant with regard to local recurrences, metastases or survival" (39). Unfortunately, these studied do not mention how many of their cases showed the presence of Bcl-2. None of our cases presented any histological evidence of malignancy in the GCTs, such as necrosis, tumor cell spindling, vesicular nuclei with large nucleoli, increased mitotic rate, a high nucleocytoplasmic ratio, or pleomorphism (39).

Does Bcl-2 immunoexpression have any other significance?

Bcl-2 protein is the product of a mitochondrial oncogene involved in the regulation of cell death. The protein acts by blocking apoptosis. Bcl-2 proteins are found in the mitochondrial membrane as it relates to the formation of pores in the membrane (40). GCTs are mainly comprised of cytoplasmic granules with the ultrastructural appearance of lysosomes, with a second cell population of "angulated bodies" resulting in a Gaucher cell–like appearance (41). Replicated basal lamina material around the granular cells suggests repeated cycles of cellular injury and repair (42). Although some mitochondrial fragments are found in the granules of GCTs (43), these organelles are not main parts of the tumor like in oncocytic tumors (44, 45). Some have related this Bcl-2 expression in oral GCTs to the prolonged life of the granular cells in oral GCTs (2).

One attractive hypothesis described pseudoepitheliomatous hyperplasia of the epidermis, which is commonly observed with GCTs (46, 47). This hyperplasia has sometimes been attributed to the influence of EGF and TGF (48). The influence of Bcl-2 in pseudoepitheliomatous hyperplasia of the epidermis has

also been suggested in cutaneous tumors other than GCTs. For instance, two cases of intense epidermal hyperplasia were presented in which the melanoma cells and the epidermis intensively expressed Bcl-2 (49). Such hyperplasia overlying melanoma, however, seems to be the exception rather than the rule regardless of whether or not Bcl-2 plays an important role in melanomas (50) and the protein can be immunostained in a high percentage of cases (51). No expression of Bcl-2 has been seen in the epithelia overlying oral GCTs (2).

Conclusions

Bcl-2 is expressed by cutaneous granular cell tumors. This information, however, seems to be more useful for the purposes of academic research than as a diagnostic or prognostic tool.

Table 1: Cases of cutaneous granular cell tumors that were examined.

Case	Gender	Age	Size (cm)	Location	CD34	S-100	HMB-45	MelanA	Bcl-2	SMA	CD68 KP1	CD68PG-M1	CKAE1-AE3	EMA	DES	CD1a	CD117	NSE
1	Male	36	2	Back	-	+	-	-	+	ND	+	ND	ND	ND	ND	ND	ND	+
2	Female	16	0.8	Left leg	ND	+	ND	ND	+	ND	+	ND	ND	ND	ND	ND	ND	+
3	Male	17	1.9	Right leg	-	+	-	-	+	-	+	+	-	-	-	-	-	+

SMA: Smooth muscle actin; EMA: epithelial mebrane antigen; DES: desmin; NSE: neuron specyfic enolase; ND: not done.

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