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Microphthalmia transcription factor and NKI/C3 expression in cellular neurothekeoma

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While the usual or myxoid-type neurothekeoma has been reasonably well established as being a tumor of neural origin, the cellular neurothekeoma remains in disputed histogenesis. We studied a series of 11 cellular neurothekeomas using paraffin immunoperoxidase staining with microphthalmia transcription factor (Mitf), NKI/C3, and S-100. The majority of the tumors in our series stained with NKI/C3 (9/11) and Mitf (9/11). All failed to stain with S-100. Furthermore, we divided our series of cellular neurothekeomas according to cytomorphology; tumors demonstrating predominantly spindled morphology, predominantly epithelioid morphology, and mixed spindle and epithelioid morphology. The two tumors that failed to stain with NKI/C3 both demonstrated predominantly spindled morphology. One of the tumors that failed to stain with Mitf showed exclusive spindled morphology, while the other showed mixed morphology (spindle and epithelioid). Two of the tumors, which stained strongly with Mitf, however, showed exclusive epithelioid morphology. This current study furthers the concept that cellular neurothekeoma is a tumor of neuroectodermal origin, and further suggests that it may express some component of melanocytic differentiation.
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Cellular neurothekeoma is an uncommon benign cutaneous neoplasm of uncertain histogenesis. Cellular neurothekeomas are often composed of spindled and epithelioid cells arranged in nests within the dermis, and sometimes resemble melanocytic neoplasia in routine histological sections. Immunohistochemical stains are helpful in discriminating cellular neurothekeoma from melanocytic neoplasia. Cellular neurothekeoma is typically reactive for NKI/C3, smooth muscle actin and neuron specific enolase and fails to stain with S-100 protein or HMB 45.

We have added anti-microphthalmia transcription factor (Mitf) to the battery of immunohistochemical stains used in our laboratory for studying possible melanocytic neoplasms The protein encoded by the microphthalmia gene is a transcription factor essential for the development and survival of melanocytes. Humans with the heterozygous mutations of Mitf have Waardenberg syndrome type II, a condition characterized by white forelock and deafness. In mice, homozygous deletion of Mitf produces complete loss of melanocytes in the skin and other organs, suggesting that Mitf is essential for the development of melanocytes.[1] Recent studies have demonstrated the antibody generated against human Mitf is reactive with metastatic malignant melanoma and appears to be a sensitive marker for melanocytes as well as melanocytic neoplasms.[2–6]

In the diagnostic evaluation of a lesion suspected to be a cellular neurothekeoma, we observed reactivity with Mitf. This led us to review the immunohistochemical profile of all the cellular neurothekeomas in our laboratory with regard to staining with Mitf, S-100 and NKI/C3.

Materials and methods

All of the cases studied were obtained from the files of Knoxville Dermatopathology Laboratory, Knoxville, TN. Only formalin-fixed paraffin embedded tissue was used. Histologically, cases were classified cytomorphologically according to whether or not they contained predominantly epithelioid cells, predominantly spindled cells, or a mixture of both. This was done in accordance with the original

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Table 1 Antibodies

Antibody	*Source*	*Dilution*
Microphthalmia transcription factor [D5]	Dana Farber Cancer Institute, Boston, MA, USA Dr DE Fisher	Undiluted hybridoma culture supernatant
S-100	DAKO	1/400
NKIC3	BioGenex	Prediluted

description of Rosati *et al*.[7] Patient clinical information regarding the lesions was collected.

Immunohistochemistry

The primary antibodies used are listed in Table 1. Paraffin blocks were sectioned into 3-μm-thick sections and mounted on Plus (+) slides. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxidase. For S-100, sections were digested with trypsin for 15 min at 37°. This was followed by a protein block (Biocare) for 10 min and incubation with S-100 for 1 h at room temperature. After rinsing with PBS, slides were incubated with a secondary antibody (goat anti-rabbit IgG, BioCare) for 15 min at room temperature, and subsequently with streptavidin-HRP for 15 min. Slides were developed with aminoethylcarbizole chromogen (BioCare).

Immunohistochemistry for Mitf and NKI/C3 was carried out in a similar fashion but with a heat-induced antigen retrieval in Reveal (BioCare) solution for 35 min. In evaluating positive staining, only nuclear staining for Mitf was regarded as valid.

Results

All 11 cases were characterized by a dermal proliferation of neoplastic cells filling and expanding the superficial and deep dermis. The lesions extended up to, but did not involve the overlying epidermis. A junctional melanocytic proliferation was not identified in any of the cases. The lesions were characterized by nests and fascicles of cells with no appreciable mucinous stroma. The nested growth pattern bore resemblance superficially to dermal nevi, however, the lack of maturation, growth into the deep dermis and poor circumscription, were consistent with cellular neurothekeoma. In addition, all cases were S-100 negative. Cytologically, six cases showed a mixed spindled and epithelioid pattern, three were mostly spindled and two were mostly epithelioid. The clinical information is summarized in Table 2.

The immunohistochemical and histological analysis of the cellular neurothekeomas is summarized in Figure 1 and in Tables 3 and 4. Nine of 11 tumors each stained with both NKI/C3 and Mitf. None stained with S-100. The two tumors, which failed to stain with NKI/C3, were mostly spindled. One of the two tumors, which was negative with Mitf, was almost exclusively spindled and the other showed an evenly mixed, spindled and epithelioid morphology. The two mostly epithelioid tumors showed strong reactivity for Mitf.

Table 2 Clinical information

Case	*Age/sex*	*Location*
1	22/F	Left shoulder
2	14/M	Right neck
3	8/M	Nasal ala
4	45/F	Thigh
5	48/F	Left nose
6	7/M	Below left nostril
7	17/F	Right lower limb
8	17/F	Posterior neck
9	19/F	Left nostril crease
10	48/F	Left wrist
11	51/F	Nose

Discussion

In 1980, Gallaher and Helwig[5] published a report on a series of benign tumors of nerve sheath origin that were dissimilar from any previously described. They[5] coined the term 'neurothekeoma' with reference to the Greek word oeke meaning sheath. Since then, these tumors have been further subdivided into myxoid or classical type and cellular variants. The cellular type of neurothekeoma was presented in two separate descriptions, the first from Italy by doctors Rosati, Fratamico and Eusebi in 1986, and a subsequent description from Barnhill and Mihm in 1990. Both papers described cellular neurothekeoma as consisting of neoplastic cells forming nests localized in the dermis or focally in the subcutaneous tissue. The nests were discrete and contained round to oval epithelioid cells with abundant eosinophilic or amphophilic cytoplasm with overlapping of usually well-defined cell membranes. The nuclei were vesicular and had dispersed chromatin patterns; however, pleomorphism and hyperchromatism were seen in some lesions.[7,8] Clinically, these tumors occur predominantly on the head and neck areas of young women.

Immunohistochemical analysis has failed to completely characterize the histogenesis or differentiation of cellular neurothekeoma. Despite the purported hypothesis of this tumor being of neural origin, it fails to stain with S-100 like its classical counterpart.[5,9–11] It has, however, been noted to stain with some neural markers, including PGP 9.5, and, more interestingly, melanocyte markers, including NKI/C3.[11,12] Other studies have shown it to be smooth muscle actin positive (see Table 5).

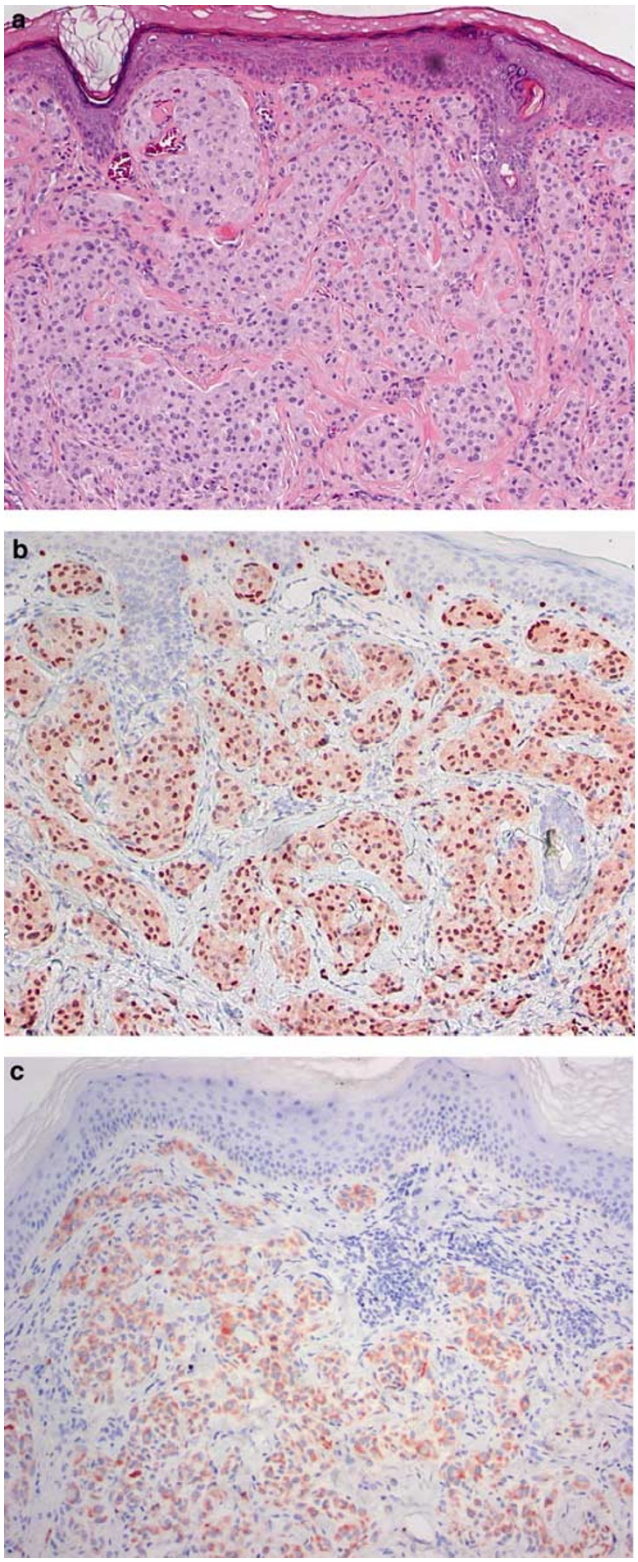

Figure 1 Staining of Mitf and NKI/C3 in cellular neurothekeoma: (**a**) hematoxylin and eosin, ×100; (**b**) Mitf, ×100; (**c**) NKIC3, ×100.

The goal of this study was to characterize the combination of Mitf and NKI/C3 expression in cellular neurothekeomas. As is evidenced in the

Table 3 Morphologic and immunohistochemical results

Case	*Type*	*Mitf*	*NKI/C3*	*S100*
1	S+E	+	+	−
2	S+E	−	+	−
3	S+E	+	+	−
4	S	Focal+	−	−
5	S	+	+	−
6	E	+	Weakly +	−
7	S	−	−	−
8	S+E	+	+	−
9	E	+	+	−
10	S+E	+	+	−
11	S+E	+	+	−

Table 4 Summary of results by morphology

	Epithelioid	*Spindled*	*Mixed*	*Total*
Mitf	2/2	2/3	5/6	9/11
NKI/C3	2/2	1/3	6/6	9/11

Results, a majority of the tumors we studied, regardless of histologic/cytologic subtype, stained strongly with Mitf and NKI/C3 markers. NKI/C3 expression has been well documented in previous studies and has consistently shown strong staining.[11] This marker, however, does not exclusively stain melanocytic markers, and also stains a variety of tumors and cell types showing neuroectodermal characteristics. To our knowledge, Mitf has been heretofore undescribed and uncharacterized in cellular neurothekeoma.

Mitf expression is not exclusive to melanocytic tumors. Mitf expression has been observed in other tumors including angiomyolipoma and other neoplasms belonging to the class of PEComas or perivascular epitheloid cell tumors.[13,14] Its expression has also been well characterized in mast cells.[15] We have found Mitf to be a reliable and helpful marker in our immunohistochemical studies of possible melanocytic neoplasia. However, one study has openly questioned the value of Mitf as a melanocyte-specific antigen.[16]

The Mitf reactivity in cellular neurothekeoma observed in our small study raises several points of interest, but fails to define the histogenesis of cellular neurothekeoma. It is possible that Mitf expression is an additional evidence that cellular neurothekeoma is a tumor of neuroectodermal origin as suggested by others, including those demonstrating it to have other neuroectodermal markers such as PGP 9.5.[11] Also possible, and most intriguing, is the concept that a cellular neurothekeoma could be in the family of the PEComas that express both smooth muscle markers and melanocyte markers.[17,18] Previous immunohistochemical studies and ultrastructural structures have demonstrated that cells in cellular neurothekeoma show features

Table 5 Summary of previous studies

Study, year (ref)	*Number of cases*	*Positive staining*	*Negative staining*
Fullen *et al*, 2003[10]	7	S-100A6, PGP 9.5	S-100
Laskin *et al*, 2000[24]	11	Colllagen type IV, SMA, calponin	S-100, GFAP, CD57, CD34, EMA, NF, p75ngfr, Factor XIIIa
Chang *et al*, 1999[25]	1	NKI/C3,SMA, NSE, synaptophysin, chromogranin	S-100, CD34, Factor XIIIa, CD68, HMB-45, CK, EMA
Zelger *et al*, 1998[21]	15	NKI/C3, KiM1p, PCNA, NSE, Factor XIIIa, SMA, vimentin	S-100, CD68, MBp, GFAP, NSE, NF, CD34, CD31, Factor VIII, ulex, desmin, myoglobin, KL1, EMA, E9
Tomasini *et al*, 1996[26]	2	Vimentin, SMA,	S-100, HMB-45, NSE, EMA, CK, desmin, CD68
Argenyi *et al*, 1995[19]	11	Collagen type IV, SMA, NSE, vimentin	S-100, CD57, GFAP, MBp, NSE, DP keratin, HMB-45, CD34, Factor XIIIa
Husain *et al*, 1994[23]	14+	MBP, EMA	A-1-ACT, MAC-387, KP-1, SMA
Argenyi *et al*, 1993[20]	4	NSE,SMA, CD57	S-100, Collagen IV, GFAP, NF, EMA, MBp, DP keratin, HMB-45, chromogranin, A1-ACT, desmin
Calonje *et al*, 1992[12]	9	NKI/C3, NSE, SMA	S-100, desmin, EMA, PGP 9.5, HMB-45
Barnhill *et al*, 1991[9]	8	Vimentin	S-100, MBP, EMA, GFAP
Barnhill and Mihm 1990[16]	5		S-100, MBP, EMA, keratin, A1-ACT, lysozyme, A1-AT,
Rosati *et al*, 1986[7]	3		S-100, lysozyme, A1-AT, MBP, Factor VIII, keratin, desmin

SMA = smooth muscle actin; GFAP = glial fibrillary acidic protein; EMA = epithelial membrane antigen; NF = neurofilament; A1-ACT = alpha 1 anti-chymotrypsin; A1-AT = alpha-1-antitrypsin; CK = cytokeratin; MBP = myelin basic protein; NSE = neuron specific enolase; E9 = antimetallothionein.
Note: Equivocal or variable staining results not included.

of Schwann cells, fibroblasts, myofibroblasts, and smooth muscle cells.[19,20] In our experience, these lesions stain in about 50 percent of cases with SMA (MC Mihm Jr, personal observation). Other studies have also shown that cellular neurothekeoma does not actually represent a variant of neurothekeoma and is in fact a tumor of another cell type.[21] Calonje *et al*,[12] suggested with their immunohistochemical and light microscopic study that cellular neurothekeoma was in fact an epithelioid variant of a pilar leiomyoma. In addition, Mitf positivity has been shown in PEComas.[17,18]

The variable immunohistochemical and ultrastructural profile of cellular neurothekeoma may be evidence of a neoplasm that undergoes variable phenotypic differentiation. This feature has been expressed *in vitro* in angiomyolipomas, which also demonstrate reactivity with Mitf. An angiomyolipoma cell line demonstrated variable terminal differentiation into smooth muscle, fat, and melanocytic cells.[22] Ultrastructural studies in cellular neurothekeoma have shown similar phenotypic variation.[19,20]

Another point of interest is that the Mitf reactivity of cellular neurothekeomas could potentially cause diagnostic confusion with melanocytic neoplasms. Spitz nevus and malignant melanoma, primary and metastatic, can show features very similar to cellular neurothekeoma. Careful histologic study and a panel of immunohistochemical markers are required for accurate diagnosis. Cellular neurothekeomas do not contain melanin pigment and do not have a junctional or intraepidermal presence. Cellular neurothekeomas are negative for S-100 and HMB-45.[23,24]

In conclusion, cellular neurothekeoma can show positivity for both NKI/C3, and Mitf, two 'melanocytic markers.' This observation should help prevent diagnostic error in classification of cellular neurothekeoma and may be evidence that cellular neurothekeoma is a type of myomelanocytic tumor in the family of perivascular epithelioid cell tumors.

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