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Microscopic intraneural perineurial cell proliferations in patients with neurofibromatosis type 1☆,☆☆

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ABSTRACT

Benign peripheral nerve sheath tumors (PNSTs) showing more than one line of differentiation (hybrid PNSTs) have been increasingly recognized, mainly due to awareness of their existence and as a consequence of increased use of immunohistochemisty during the last decade. Two recent studies suggested overrepresentation of hybrid tumors among benign PNSTs in patients with neurofibromatosis type 1 (NF-1). This study was performed to assess the presence of perineurial cells in microscopic (early) neurofibromatous lesions and normal-looking peripheral nerves in specimens from 5 patients with NF-1 using markers of perineurial cell differentiation (epithelial membrane antigen, claudin-1, and glucose transporter 1). In 2 patients, multiple normal looking nerve fibers as well as hypertrophied nerves and microscopic tumor nodules showed variable intraneural perineurial cell proliferations that frequently occupied the whole nerve fascicle resulting in multiple microscopic reticular perineurioma-like nodules (microscopic hybrid neurofibromatosis/perineuriomatosis). None of the cases showed the onion skin pattern of intraneural perineurioma. However, other nerve fibers within the same specimens showed normal compact rim of perineurium without any detectable intraneural perineurial cells. Both patients had concurrent multiple larger PNSTs (plexiform neurofibromas, hybrid neurofibroma/perineurioma and lesions with features intermediate between the 2 types). One specimen harboring high-grade malignant PNST and 2 specimens with large solitary neurofibromas displayed no intraneural perineurial cells. These observations suggest that intraneural perineurial proliferations are part of the early lesions in the setting of constitutional *NF-1* inactivation and support the concept of pure and hybrid perineuriomatous lesions as novel member of the spectrum of PNSTs in NF-1.

1. Introduction

Benign peripheral nerve sheath tumors (BPNSTs) differentiate similar to the normal cellular constituents of the peripheral nerve sheath [1]. Accordingly, neurofibroma, schwannoma, and perineurioma represent the major subtypes of BPNSTs. While neurofibroma is composed of an admixture of Schwann cells, perineurial fibroblasts, scattered axons and a few perineurial cells representing remnants of the nerve sheath, schwannomas on the other hand are considered to display uniform Schwann cell differentiation. Nevertheless, several studies have demonstrated the presence of scattered neuroaxonal fibers within soft tissue schwannoma [2] and perineurial cells have been demonstrated within diverse PNSTs [3]. Furthermore, the traditional classification of the individual subtypes of BPNSTs has been challenged by recognition of more than one line of differentiation in some BPNSTs where 2 cell populations were prominent enough to justify a diagnosis of a biphasic neoplasm with hybrid differentiation [4]. Since then, variable combinations of 2 or more cell types of the peripheral nerve sheath have resulted in recognition of diverse variants of hybrid BPNSTs, the most well characterized of them are hybrid neurofibroma/perineurioma [5], hybrid schwannoma/perineurioma [6,7], hybrid granular cell tumor/perineurioma [8], hybrid schwannoma/neurofibroma [4], hybrid schwannoma/neurofibroma/perineurioma [9], and more recently, hybrid perineurioma/neurothekeoma [10].

Only 2 recent studies have addressed the presence of hybrid differentiation in BPNSTs occurring in the setting of neurofibromatosis type 1 (NF-1; von Recklinghausen disease) [11,12]. However, precursor lesions of these hybrid BPNSTs have not been described. Furthermore, 2 cases of soft tissue perineurioma have been documented in patients with proven NF-1 [13,14]. Molecular studies in one of these NF-1-associated perineuriomas showed absence of *NF2* gene alterations characteristic of sporadic soft tissue perineurioma [15,16], thus raising the hypothesis, that perineurial cell proliferations might be part of the spectrum of nerve sheath lesions caused by constitutive inactivation of *NF-1* [14]. Of note, the specimen of that patient contained microscopic foci of perineurial cells extending from nerve sheath (perineurium) into the surrounding soft tissue, suggesting that such perineurial cell proliferations might represent the precursors of perineurial cell tumors in the setting of NF-1 [14]. The aim of this

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study was to examine the presence of perineurial cell proliferations in resections from NF-1 patients.

2. Material and methods

The resection specimens from 5 patients with NF-1 who underwent removal of benign or malignant PNSTs have been screened for the presence of hybrid PNSTs on hematoxylin and eosin (HE) stained slides. After HE screening, representative 1 to 4 blocks were chosen from each case based on presence of normal looking nerve fibers at the tumor/specimen periphery and presence of features suggestive of perineurial cells. Recuts from these blocks were then assessed by immunohistochemistry for perineurial cell markers: epithelial membrane antigen (EMA, clone E29, 1:200, microwave pretreatment, Dako, Hamburg, Germany), glucose transporter 1 (GLUT-1, polyclonal, 1:200; ThermoScientific, Fremont, CA), claudin-1 (polyclonal, 1:100; Zytomed, Berlin, Germany), and protein S-100 (polyclonal, 1:2500, Dako). Staining was performed on freshly cut 3-μm paraffin sections using a fully automated slide preparation system "Benchmark XT System" (Ventana Medical Systems Inc, Tucson, AZ). No specialized staining enhancement protocol was used for EMA.

3. Results (Table 1)

All 5 patients have clinically proven NF-1 (each fulfilled >2 major criteria according to the National Institutes of Health consensus conference on NF-1 [17]). Four of them had myriads of cutaneous perineuriomas distributed all over the whole body (Fig. 1A). One patient who was diagnosed with a parapharyngeal malignant peripheral nerve sheath tumor (MPNST) arising in a large plexiform neurofibroma presented with extensive microscopic involvement of the cervical soft tissue and the thyroid gland (Fig. 1B). Only a few of the numerous microscopic neurofibromas examined from this patient (>40 microscopic lesions) showed unequivocal features suggestive of a hybrid neoplasm on HE-stained slides (Fig. 1C and D). However, immunohistochemical stains showed fine reticular networks of perineurial cells within several nerve fascicles that extended variably into the substance of individual nerve fascicles occasionally obliterating the normal nerve tissue corresponding to hybrid neurofibroma-perineurioma lesions (Fig. 2A-D). The perineurium surrounding the nerves showed no significant thickening or proliferations extending into the surrounding soft tissue or along adjacent vessels. Microscopic and gross neurofibromatous nodules showed a mixture of hybrid and non-hybrid lesions. Some plexiform neurofibromas showed hybrid features limited to single lobules. The second patient has similar lesions found in lymph node dissection for leg melanoma. The neurofibromas in this patient were interpreted on positron emission tomography/computed tomography as suspicious for melanoma metastasis. Of note, features of intraneural perineurioma, that is, onion skin-like proliferations of perineurial cells around neuroaxons were not seen in any of the lesions. Four specimens from the remaining 3 patients lacking microscopic or plexiform neurofibromatosis (3 large solitary neurofibromas and one MPNST) did not show intratumoral or intraneural perineurial cell proliferations.

4. Discussion

Traditionally, BPNSTs in patients with proven NF-1 have been classified as neurofibromas based on their overall histological appearance in HE-stained sections. The presence of scattered perineurial cells within sporadic neurofibroma is well known and usually considered to represent entrapment of residual nerve sheath elements. However, as the concept of single-line-differentiation in PNST has been challenged by the observation of tumors featuring equal amount of 2 distinctive cell populations [4], awareness of the existence of dual differentiation in PNST (hybrid tumors) has increased and is positively influenced by the availability of relatively specific and highly sensitive perineurial cell markers. As a consequence, both sporadic and NF-1 associated hybrid PNSTs have been increasingly reported during the last decade [11,12,18].

Tow recent studies have demonstrated that hybrid differentiation is rather common in benign and also in rare malignant PNSTs if immunohistochemistry is used as an adjunct tool to identify perineurial cells [11,12]. In the current study, histologically normal looking nerve fibers in resections specimens harboring PNSTs from NF-1 patients were analyzed for their perineurial cell contents. Several small to medium sized nerve fibers showed prominent reticular networks of EMA/claudin-1/GLUT-1-positive perineurial cells. This occurred in 2 patients with myriads of microscopic lesions that at least in part qualified as neurofibromatosis/perineuriomatosis and occurred in conjunction with other-type PNSTs including plexiform neurofibroma/tosis and larger hybrid neurofibroma/perineurioma. It is noteworthy that larger solitary neurofibromas and 2 MPNSTs did not contain increased perineurial cells. Remarkably, small nerves within specimens without microscopic plexiform neurofibromas/neurofibromatosis did not contain intraneural perineurial cell lesions. A variable degree of perineurial thickening was seen at the periphery of larger plexiform neurofibromas occasionally with some irregular extension into the adjacent neurofibromatous component. Of note, some tumor nodules showed binodular pattern with closely associated perineuriomatous and neurofibromatous proliferation.

Although perineurial cell neoplasms (perineuriomas) are generally not considered part of the PNST spectrum in NF-1, 2 cases of well documented soft tissue perineurioma affected patients with NF-1 [13,14]. one of these cases showed foci of perineurial cell proliferations encasing minute nerve twigs and extending along adjacent small vessels for some distance [14]. Absence of *NF2* gene alterations in that case supported a perineurial proliferation in the context of the underlying NF-1, as most of perineuriomas harbor *NF2* gene alterations [15,16].

Consistent with these above observations, prominent perineurial cell population was detected immunohistochemically within otherwise unremarkable nerve substance of several peripheral nerve fibers in the soft tissue surrounding PNSTs from patients with NF-1 in the

Table 1
Clinicopathological features of NF-1 patients assessed for perineurial cell proliferations

No	Age/ Gender	Site of specimen	Histological diagnosis	Perineurial cells in PNSTs	Perineurial lesions in nerves
1	41 F	Mediastinum para-esophageal	Solitary neurofibroma	None	Very few, scattered
2	71 F	Inguinal node dissection for melanoma (plexiform NFs mistaken for metastasis)	Multiple (>10) plexiform neurofibromata, hybrid neurofibroma/perineuriomas	Variable, some qualified as hybrid tumors	Prominent in many nerves
3	49 F	Resection for head & neck MPNST	MPNST	None	None
4	26 F	13 neurofibromata from all over body	Multiple neurofibromata	Scattered within 2 examined neurofibromata	Absent in >10 nerve fascicles
5	54 F	Parapharyngeal + thyroid, MPNST	MPNST within plexiform neurofibroma, microscopic hybrid neurofibromatosis/ perineuriomatosis	Prominent in diffuse minute nodules = hybrid neurofibromatosis/ perineuriomatosis	Prominent in >50% of nerves.