Trichilemmal carcinoma with neuroendocrine differentiation

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Summary

We report a 12-mm nodular, cream-coloured skin lesion that appeared on the left nasal ala in an 81-year-old man. This trabecular infiltrative tumour showed keratin microcysts, stromal hyalization, cytoarchitectural malignancy features, colonizing melanocytes, and immunoexpression of epithelial membrane antigen, cytokeratin 15/20, chromogranin, synaptophysin and CD56. To our knowledge, this is the first documented case of a trichilemmal carcinoma with neuroendocrine differentiation and melanocyte colonization, which is suggested by the trabecular growth pattern and requires immunohistochemical confirmation. The colonization of the epithelial nests by nonatypical dendritic or spindle melanocytes is a clue to morphological recognition of pilar neoplasms, along with the presence of stromal induction (CD34-positive peritumoral spindle cells), catagen-like apoptotic bodies, calcifications, keratin microcysts and cell balls.

Skin adnexal tumours are rare entities causing diagnostic dilemmas in histopathological practice, for which the pattern recognition is diagnostically essential.¹ Helpful clues include the presence of intracellular and intercellular lumina containing diastase-resistant, periodic-acid–Schiff (PAS)-positive material (ductal tumours), vacuolated epithelial cells with nuclear indentations (sebaceous tumours) and pilar elements (tumours with hair follicle differentiation). Adnexal neoplasms with pilar differentiation are the most elusive, often diagnosed as basal cell, squamous cell or undifferentiated carcinomas. The pilar differentiation is suggested by the presence of PAS-positive hyalinized stromal changes around the epithelial nests, keratinous microcysts and/or cell 'balls' with or without peripheral nuclear palisading, and changes in the eosinophils and clear cytoplasm.^{1,2} Features suggestive of pilar differentiation and malignancy criteria have been reviewed in detail elsewhere.^{3–6}

The presence of melanocytes within various tumours has been reported, but they are almost always present in tumours with pilar differentiation.² The terms 'pigmented' or 'melanocyte-containing' tumours have been used to describe 'new' entities or variants that occur when the number of melanocytes is increased in an otherwise conventional and well-recognized entity.⁷

We present a trichilemmal carcinoma with neuroendocrine differentiation and melanocyte colonization, discussing useful features to recognize its differentiation. Features helpful to diagnosis of skin adnexal tumours with epithelial pilar differentiation are outlined, in particular for cases with other lines of differentiation that may cause further diagnostic difficulties.

Report

The patient was an 81-year old man, who presented with a 12-mm nodular, cream-coloured skin lesion on the left ala of nose of unknown duration. The clinical diagnosis was of basal cell carcinoma. The specimen was routinely processed, inking the margins and serially sectioning the 20-mm area containing the lesion, which was 5 mm from the closest lateral margin.

The tumour showed infiltrative growth in trabeculae and strands, and contained keratin cysts and hyalinized

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Figure 1 Adnexal carcinoma with divergent differentiation. (a) An infiltrative margin, no connections with the superficial epidermis, and no abnormalities in the surrounding skin. (b,c) The neoplasm shows a trabecular growth pattern, and several features of pilar differentiation, such as hyalinized stroma and atypical cells with prominent nucleoli and mitotic figures along with (d) eosinophilic 'cell balls'. Haematoxylin and eosin; original magnification (a) $\times 25$, inset $\times 100$; (b–d) $\times 400$.

stroma (Fig. 1). Cytologically, there was marked nuclear pleomorphism, small but distinct nucleoli and focal clear-cell changes. Numerous mitotic figures including abnormal forms were evident as were focal areas of necrosis. Another sparse population of spindle-shaped melanocytes was also noted within the tumour islands.

Sections were mounted on positively charged microscope slides (Superfrost Plus, Fisher Scientific, NJ, USA) and baked at 60 °C for 2 h. After routine dewaxing, rehydration and endogenous peroxidase quenching, the sections were sequentially incubated with polyclonal horse serum (1 : 100 dilution; Dako, Glostrup, Denmark), the corresponding primary antibody (4 µg/mL for all monoclonal antibodies; Dako) overnight at 4 °C, biotinylated antimouse antibody (1 : 200 dilution; Dako) and peroxidase-labelled avidin–biotin complex (1 : 100 dilution, Dako). All incubations were performed in a moist chamber at room temperature. The reaction was developed under microscopical control, using 3,3'-diaminobenzidine tetrahydrochloride as chromogen (Sigma, St. Louis, MO, USA), and the sections counterstained with haematoxylin. Both positive (reactive lymph node) and negative (omission of primary antibody) controls were simultaneously run.

Tumour cells were positive for epithelial membrane antigen (EMA) (75–80%); CD56, synaptophysin, chromogranin (25–30%); and cytokeratins 15 and 20 (80%+, paranuclear dot pattern) (Fig. 2). The spindle melanocytes showed positive staining with S100, HMB45, and melanA (5%); a small number of CD1a-

positive dendritic Langerhans' cells (1–3%) were present. The peritumoral stroma was CD34-positive.

The tumour had an infiltrative pattern with foci of necrosis, pleomorphic cells and atypical mitoses, suggesting a malignant neoplasm, and showed evidence of pilar differentiation, i.e. keratin cysts, peritumoral hyalinized stroma with CD34+ spindle cells, and tumour cells positive for CK15, CK20 and EMA (the latter previously reported occurring focally).³ Tumour cells were focally positive for neuroendocrine markers (chromogranin, synaptophysin and CD56). The diagnosis was trichilemmal carcinoma with neuroendocrine differentiation.

Neuroendocrine differentiation has not previously been reported in pilar neoplasms, with the exception of trichogerminoma.⁸ This neuroendocrine differentiation has been correlated with vascular endothelial growth factor and transforming growth factor- α immunorexpression in carcinomas. These two angiogenic factors may aid the neovascularization of carcinomas, and their increased expression in tumour-associated neuroendocrine cells may contribute to a more aggressive phenotype in several types of tumour.⁹

Primary cutaneous neuroendocrine (Merkel cell) carcinomas mainly present as dermal and subcutaneous masses in the sun-exposed skin of elderly patients. They show generally monomorphic cells with foci of pronounced pleomorphism. These tumours are heterogeneous and can occur in intimate association with carcinomas (particularly squamous and eccrine types).¹⁰

Figure 2 Immunohistochemical profile of adnexal carcinoma with divergent differentiation. Tumour cells show cytoplasmic expression for (a) cytokeratin 15 and (b) chromogranin A. Tumour nests show (c) numerous MelanA-positive intraepithelial dendritic cells with hyperchromatic nuclei and (d) occasional CD1a-positive Langerhans cells, predominantly stromal). The stroma surrounding the tumour nests is CD34 positive (inset). Original magnification (a–c) $\times 200$; (d) $\times 100$, inset $\times 100$.

Table 1 Differential diagnosis of trichilemmal carcinomas with divergent differentiation.

Categories	Conditions	Key diagnostic features
With neuroendocrine differentiation	Merkel cell carcinoma	Infiltrative trabecular growth Differentiated and intraepithelial malignant components can be present Irregularly distributed chromatin, no nucleolus Diffuse expression of neuroendocrine and neuronal markers Occasionally, expression of divergent differentiation markers in malignant cells (e.g. melanocytic, glandular)
	Metastatic neuroendocrine carcinoma	Infiltrative growth No connections with superficial or adnexal epithelia Irregularly distributed chromatin, no nucleolus Frequent lymphovascular invasion Diffuse expression of neuroendocrine markers Expression of tissue-specific markers (e.g. TTF1, PSA)
	Neuroendocrine differentiation in primary skin carcinomas (excluding basal cell carcinoma)	Infiltrative growth Nuclear (nuclear enlargement and distinct nucleolus) and differentiation features of specific tumours (e.g. ductal, squamous cells) Frequent connections with superficial or adnexal epithelia Rare lymphovascular invasion Focal expression of neuroendocrine markers
With melanocytic component	Pigmented pilar-type basal cell carcinoma	Infiltrative growth pattern, deep Connections with superficial epithelium Basaloid cells with focal evidence of pilar differentiation (hyaline cytoplasm, "cell balls", catagen-like apoptotic bodies, calcification, D-PAS-positive stromal induction, peritumoral CD34-positive spindle cells). Retraction artifact at epithelial-stromal junction Pigmentation/colonization by non-tumoral melanocytes

D-PAS, diastase-resistant periodic-acid–Schiff; PSA, prostate-specific antigen; TTF1, thyroid transcription factor 1.

Merkel cell carcinomas have also been reported associated with a trichilemmal cyst, showing pagetoid spread into the trichilemmal epithelium and other

cutaneous malignancies.¹¹ The association of Merkel cell carcinomas with other adnexal lesions and their dermal location strengthen the hypothesis of an origin

from pluripotent stem cells of adnexal epithelium.¹¹ In the case reported here, malignant cells showed a predominant pilar differentiation with only focal neuroendocrine features (Table 1). Abnormal differentiation has been inversely correlated with malignancy and precancerous lesions; progressive undifferentiation is often associated with advanced tumour stages and divergent differentiation is not unusual in a variety of poorly differentiated malignant tumours (e.g. breast ductal carcinoma, gastrointestinal adenocarcinomas or desmoplastic small round cell tumour). The neuroendocrine differentiation is important, because these tumours have been reported to be resistant to chemotherapy.

The presence of melanocytes in epithelial tumours is relatively rare in cutaneous or extracutaneous neoplasms. The systematic presence of nonatypical spindle melanocytes in pilar neoplasms questions the separation of pigmented pilar neoplasms as independent entities.^{2,7} These conditions probably represent extreme forms of melanocyte colonization of epithelial pilar tumours and do not merit creation of 'new' entities such as 'pigmented' or 'melanocyte-containing' tumours.² The most important issue is to determine if the melanocytic component shows evidence of dysplasia or malignancy using standard criteria.^{12,13} Dysplastic and malignant changes may occur in the melanocytic component of epithelial pilar neoplasms and would explain the description 'biphasic neoplasms'.^{7,14} Melanocytes are found within the epithelial nests of epithelial pilar tumours (both benign and malignant),² and can be used as a clue to the recognition of this differentiation in the absence of clinical pigmentation or epithelial cell melanin during histological examination.² Melanocytes are rarely identified in other cutaneous epithelial tumours, and are normally associated with clinical and/or histological pigmentation.

In conclusion, neuroendocrine differentiation in trichilemmal carcinomas is suggested by a trabecular growth pattern and requires immunohistochemical confirmation. The colonization of the epithelial nests by nonatypical dendritic/spindle melanocytes is a clue to the morphological recognition of pilar neoplasms, along with the presence of stromal induction, catagen-like apoptotic bodies, calcification, keratin microcysts and cell balls. This feature lends the tumours a heterogeneous appearance, containing tumour cells and dendritic cells that may be pigmented.

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