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Merkel cell differentiation in trichoblastoma

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Abstract Four cases of trichoblastoma rich in Merkel cells (MCs) are reported. They occurred in two men and two women, with ages ranging from 58 to 76 years (mean 67.5 years). MCs were detected immunohistochemically with antibodies to keratin 20, chromogranin A and neuron-specific enolase (NSE). In an attempt at better definition of the nature and role of MCs in trichoblastoma, the distribution of MCs in normal adult and fetal skins obtained at autopsy was studied. In addition, ten cases of sebaceous naevus of Jadassohn (NSJ) were evaluated along similar lines. MCs made up 2–20% of the tumour cells in trichoblastomas; they were present in normal fetal skin and were rare in normal adult skin. All but one of the cases of NSJ showed numerous positive cells in the epidermal component of the lesion with all three antibodies. Six basal cell carcinomas and one syringocystadenoma papilliferum associated with NSJ were negative with keratin 20, chromogranin A and NSE antibodies, whereas a minute trichoblastoma arising against the same background was positive for these markers. Hair follicle cell tumours may recapitulate the skin embryogenesis, as numerous MCs are present in fetal follicles, but only occasional such cells are seen in adult skin.

Key words Merkel cell · Trichoblastoma · Fetal skin · Normal adult skin

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Introduction

Trichoblastomas are rare tumours of the skin, which may occur as solitary dermal neoplasms or in association with sebaceous naevus of Jadassohn (NSJ) [1]. These were subdivided by Headington [8] into four different categories, trichoblastoma, trichoblastic fibroma, trichogenic trichoblastoma and trichogenic myxoma, according to the appearance of the stromal component. Subsequently, Ackerman et al. [1] proposed the unifying term of trichoblastoma to encompass cutaneous tumour types with circumscribed margins and a predominance of follicular germinative cells.

It has been reported that trichoblastoma can be associated with NSJ [1], and these lesions have been shown to be composed of follicular germ cells intermingled with Merkel cells (MCs) [21].

The aim of the present study is to describe four cases of trichoblastoma rich in Merkel cells (propositus cases). In addition, and in an attempt at better definition of the nature and the role of MCs in these lesions, the presence and distribution of MCs were evaluated in normal fetal and adult skins and also in cases of NSJ (reference cases).

Materials and methods

Propositus cases

The cases studied were obtained from the files of the Institutes of Anatomic Pathology of the University of Bologna at Bellaria Hospital and of Modena (1 case).

All tissue specimens were fixed in 4% formaldehyde and embedded in Paraplast. Sections were stained with haematoxylin and eosin (H&E), PAS with diastase control, and Alcian blue. One case was studied ultrastructurally (case 1) after retrieval from formalin-fixed tissue. The material was post-fixed in osmium tetraoxide, dehydrated, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and viewed with a Zeiss M10 electron microscope.

Immunohistochemistry was performed in all cases using the avidin–biotin–peroxidase complex [9]. Commercially available antibodies were used. Their sources and dilutions are listed in Table 1.

Table 1 Antibodies used for immunohistochemistry (*NSE* neuron specific enolase)

Antibody	Source	Clone	Dilution
Keratin 20	Dako, Glostrup, Denmark	KS20.8	1:40
Chromogranin A	BioGenex Ca USA	LK2H10	1:200
NSE	BioGenex	N3	1:400
Keratin (wide spectrum)[a]	Dako	MNF116	1:100
Smooth muscle actin[a]	Dako	1A4	1:100

[a] Antibodies used only in propositus cases

Table 2 Clinico-pathological features of the propositus cases with trichoblastoma (*FU* follow-up, *A&W* alive and well)

No. of Cases	Sex	Age	Site	Size (cm)	F.U. (months)
1	Female	61	Arm	1.5	17 A&W
2	Female	76	Neck	3.5	21 A&W
3	Male	75	Back	4	21 A&W
4	Male	58	Thigh	3	36 A&W

Reference cases

Five fetal skin samples (20–22 weeks of gestation) were obtained at autopsy from different body sites (arm, leg, abdomen, thorax, scalp, and zygomatic region). Post-mortem tissues were also obtained from the skin of ten adult patients who had died of a variety of diseases without cutaneous manifestations. The sites studied paralleled those examined for fetal skin (with the exception of the zygomatic region, from where none was taken).

Ten cases of NSJ were also specially selected. Eight of these cases were associated with neoplasms: basal cell carcinoma (6 cases), syringocystadenoma papilliferum (1 case), and a minute trichoblastoma (1 case).

In normal fetal and adult skins a semiquantitative analysis of keratin 20-, chromogranin A- and NSE-immunoreactive cells was performed in the epidermis and in the follicular epithelium. The results were listed as positive (+) when >1 in 50 basal cells was immunoreactive either in the epidermis and in the follicular epithelium, focally positive (+/–) when <1 cell in 50 basal cells was present, and negative (–) when immunoreactive cells were absent.

Results

Propositus cases

The relevant clinical data are summarized in Table 2. Two tumours (cases 2 and 4) shelled out during the surgical procedure, while two others (cases 1 and 3) were removed together with the overlying skin. Their cut surface was whitish and homogeneous.

Histologically all cases showed similar features, and therefore these will be described together.

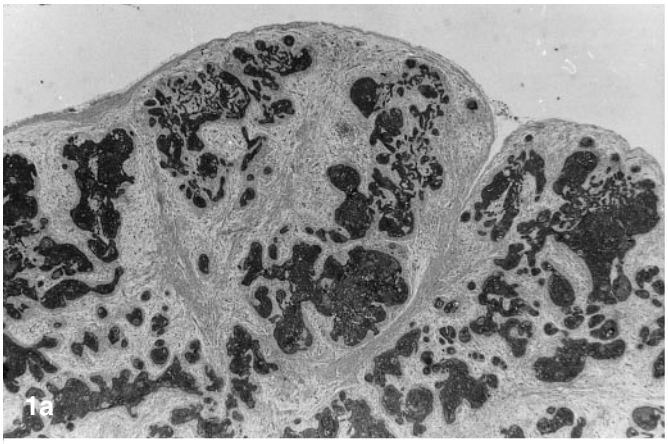
The tumours were well circumscribed and not connected to the overlying epidermis, which showed slight acanthosis and hyperkeratosis (Fig. 1A, B). They were composed of sheets and nests of neoplastic cells, interconnected by thin strands that in some areas formed a definite trabecular pattern (Fig. 2A, B). The former cells had large round nuclei and scanty cytoplasm. Some of the sheets and nests showed central groups of cells with abundant pale eosinophilic cytoplasm reminiscent of squamous eddies surrounded by a peripheral palisading (Fig. 3). Follicular differentiation was present in the form of minute follicular germs and papillae. The stroma displayed numerous plump fibroblasts and, in case 3, it showed prominent myxoid features. No clefts between the tumour nests and the stroma were noted. Numerous mitoses were present in the epithelial component, some of them with atypical features. No recurrences or metastases developed after a follow-up ranging from 17 to 36 months (mean 24 months).

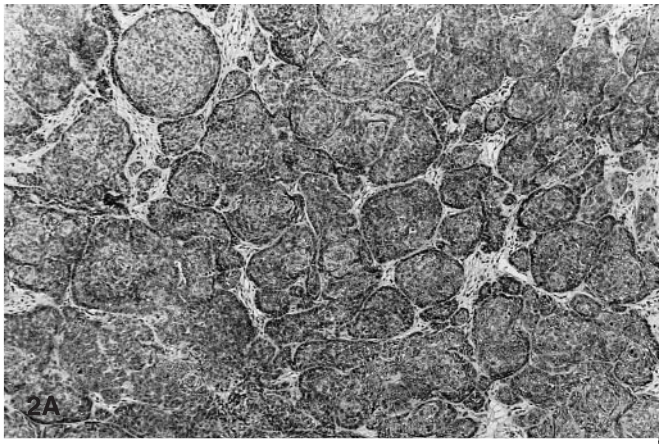
Electron microscopically (case 1), there were two distinct cell populations: keratinocytes with numerous bundles of tonofilaments, and cells with a low-density cytoplasmic matrix containing sparse intermediate filaments consistent with keratin. Cells containing numerous neuroendocrine-type granules were also visible. These ranged from 60 to 120 nm in diameter. They showed a dense core surrounded by a pale halo and they were membrane-bound (Fig. 4A). Some of these neuroendocrine-type granules aggregated in long cytoplasmic prolongations of the tumour cells.

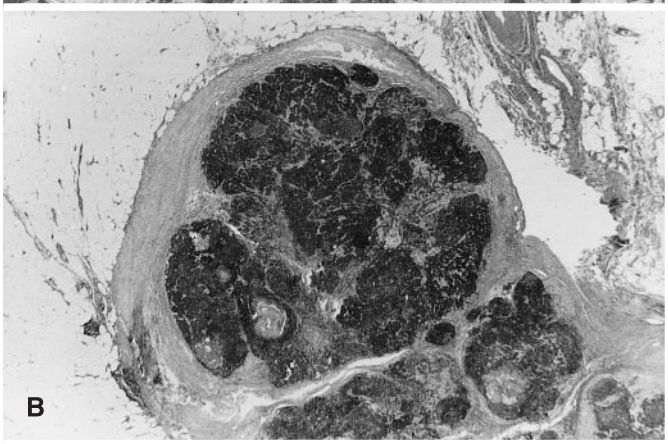
The results of the immunohistochemical study are summarized in Table 3. All neoplastic cells were intensely positive with the antibody against wide-spectrum keratin and negative with smooth muscle actin. Antibodies against keratin 20, chromogranin A, and NSE decorated a population of cells located mostly in the centre of the tumour sheets and nests. These cells often had a triangular shape, with numerous dendritic cytoplasmic projections (Fig. 4B–D). The immunoreactive elements ranged from 2% to 20% of the total neoplastic population in the various cases. It was not possible to distinguish the immunoreactive cells from those that were negative in routinely stained sections. The epidermis overlying the tumours in cases 1 and 3 did not contain cells immunoreactive for keratin 20, chromogranin A or NSE.

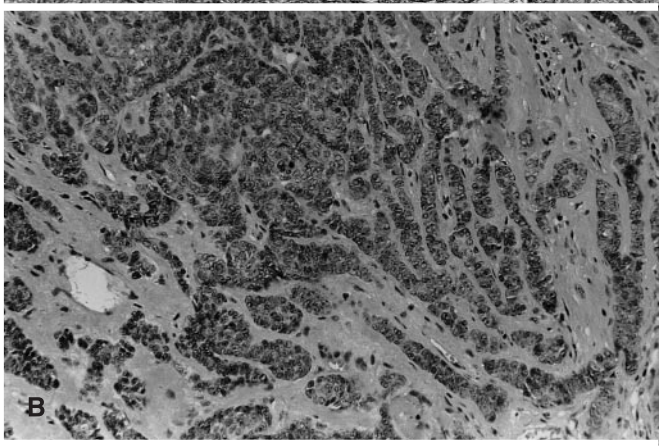
Reference cases

The results for fetal skin are summarized in Tables 4 and 5. All cases showed a variable number of positive cells located in the basal layer of the epidermis and the follicular structures. Nineteen sections out of 120 obtained from different body sites from the five cases contained









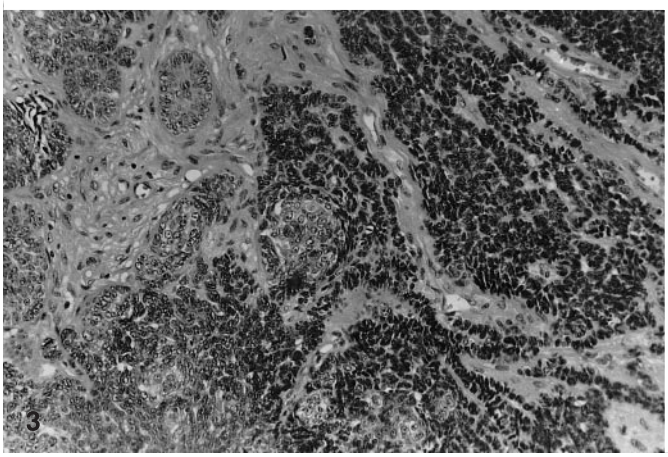


Fig. 1A, B Trichoblastoma. The tumour has well circumscribed tumour cell nests. H&E **A** Case 3. ×20 **B** Case 4. ×40

Fig. 2A, B Trichoblastoma in case 1. The tumour is composed of nests and sheets of cells (**A**) interconnected by thin trabeculae (**B**). H&E, **A**×100, **B**×200

Fig. 3 Trichoblastoma in case 1. The tumour nests and sheets are composed of two types of cells: with scanty cytoplasm, and with abundant pale cytoplasm reminiscent of squamous eddies. H&E, ×200

Table 3 Immunohistochemical results, expressed as percentages of positive cells (*CgA* chromogranin A, *NSE* neuron specific enolase, *SMA* smooth muscle actin)

Antibody	Case 1	Case 2	Case 3	Case 4
Keratin 20	20%	15%	15%	10%
CgA	10%	5%	5%	2%
NSE	10%	20%	10%	5%
SMA	–	–	–	–
Keratin[a]	90%	90%	90%	90%

[a] Keratin wide spectrum

>1 positive cell for every 50 keratinocytes (range 2–5), both with keratin 20 and with chromogranin A antibodies when epidermis and follicular epithelium were considered. NSE stained a larger number of cells along the basal layer (Fig. 5A, B). No predilection for particular sites was seen.

In adult skin only one specimen (taken from the scalp) showed rare cells located in the basal layer of the epidermis that were positive for keratin 20, chromogranin A, and NSE. Negative results were obtained in all the other skin samples examined for all antibodies tested.

All but one NSJ showed a variable number of cells that were immunoreactive with keratin 20, chromogranin A, and NSE antibodies. These cells were located in the basal layer above the hyperplastic rete ridges and in the primitive hair follicles. All basal cell carcinomas (six cases) and syringocystadenoma papilliferum (one case) associated with NSJ lacked immunoreactive cells.

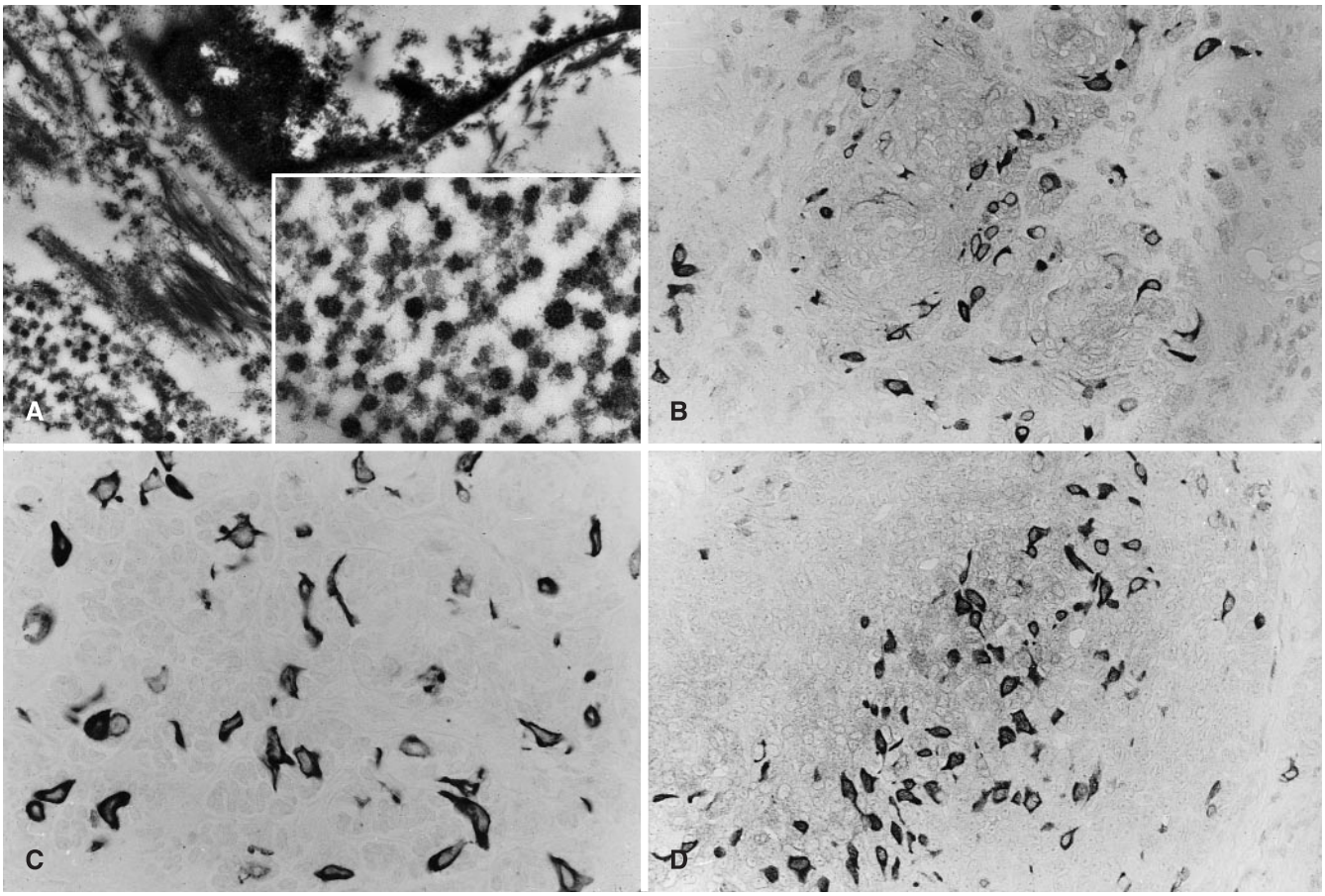


Fig. 4A–D Trichoblastoma in case 1. Electron microscopic appearance of a keratinocyte with numerous bundles of tonofilaments adjacent to a Merkel cell **A**. Small neuroendocrine-type granules showing sharp borders in a Merkel cell (*inset*). Merkel cells present inside the tumour nests show a triangular shape and exhibit occasional cytoplasmic prolongation at immunohistochemical level. A×28000, *inset*×56000; **B** chromogranin A, ×400; **C** neuron-specific enolase, ×600; **D** keratin 20, ×400

Table 4 Immunophenotype of Merkel cells (MCs) in normal fetal epidermis from five cases (K_{20} Keratin 20)

Site	K_{20}	CgA	NSE
Leg	1\5	2\5	3(1[a])\5
Back	1[a]\5	2(1[a])\5	3(2[a])\5
Thorax	1[a]\5	3(2[a])\5	3(2[a])\5
Arm	3(1[a])\5	2[a]\5	2[a]\5
Zygomatic area	3(1[a])\5	3(1[a])\5	3(1[a])\5
Scalp	1\5	3(1[a])\5	3(2[a])\5

[a] Cases showing more than one MC to every 50 basal cell

However, the single case of NSJ that was associated with a minute trichoblastoma showed numerous tumour cells that were immunoreactive with the three antibodies, similar to those seen in the propositus cases (Fig. 6A, B).

Table 5 Immunophenotype of MCs in the follicular epithelium of human fetal skin from five cases

Site	K20	CgA	NSE
Leg	1\5	2\5	3(2[a])\5
Back	1[a] \5	2(1[a])\5	3[a]\5
Thorax	1[a] \5	3\5	3[a]\5
Arm	1(1[a])\5	1[a]\5	2[a]\5
Zygomatic area	2(1[a])\5	1[a]\5	2[a]\5
Scalp	2(1[a])\5	1\5	4(1[a])\5

[a] Cases showing more than one MC every 50 basal cell of the follicular epithelium

Discussion

The lesions we studied were typical examples of trichoblastoma [1]. They presented as well-circumscribed dermal nodules composed of nests and sheets of basaloid cells showing peripheral palisading. These were surrounded by an exuberant fibroblastic stroma, which in one case had prominent myxoid features. In some areas the neoplastic cells showed hair follicle differentiation, including minute follicular germs and papillae. In all these cases we were able to demonstrate numerous tumour cells immunoreactive for keratin 20, chromogranin A, and NSE. In addititon, electron microscopic examination of case 1 revealed endocrine-like granules. These

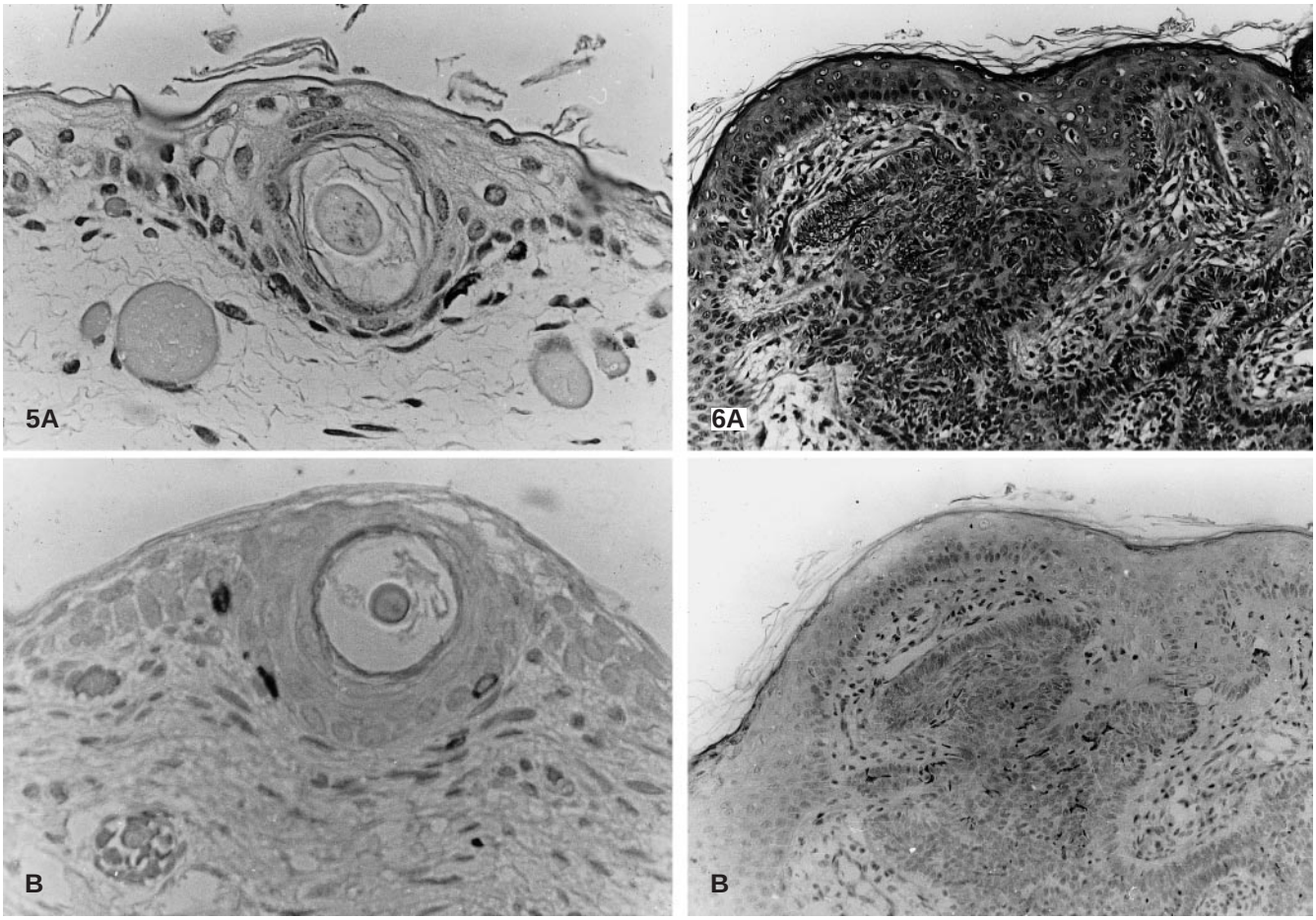


Fig. 5 **A** Merkel cells positive for chromogranin A and **B** neuron-specific enolase are present in the follicular epithelium of fetal skin. ABC Immunoperoxidase, **A**×400, **B**×600

Fig. 6 **A** Naevus sebaceus of Jadassohn. Minute trichoblastoma showing peripheral palisading. H&E, ×200 Numerous Merkel cells are present. ABC immunoperoxidase **B** chromogranin A, ×150

combined features are clearly indicative of the presence of neuroendocrine differentiation in these tumours [11, 17, 19, 20].

Interestingly, the same immunohistochemical features were seen in a minute trichoblastoma associated with NSJ. Our findings are similar to those reported by Schulz and Hartschuh [22], who found that 42% of their 36 cases of trichoblastomas contained various numbers of MCs. These same authors had previously reported on nine cases of trichoblastomas associated with NSJ, which also contained MCs [21].

We found easily detectable MCs in the basal layer of the epidermis and outer root sheath of follicular epithelium in all of the normal fetal skins we examined. Conversely, these cells were absent from normal adult skin (with the exception of a single specimen, in which rare cells were detected). This pattern parallels that observed in normal fetal lung, in which the endocrine cells are easily detectable in the fetal period and progressively decrease in number, to become almost non existent in adult life [10].

Moll et al. 1990 [18] found a considerably higher number of MCs in normal adult skins than in the present study (from a mean of 10.8 MCs per square millimetre in the skin of the arm to a mean of 21 MCs in the skin of the trunk). However, it should be noted that their cases were in patients who had undergone excision of skin tumours. Many of these had arisen in skin affected by sun damage, a condition known to be associated with an increase in MCs [10, 16]. Furthermore, they used keratin 18 antibody to localize MCs, a marker later regarded by the same authors as not specific for this cell type [20]. Lacour et al. [13] mapped MCs in human normal adult skin anatomically using an antibody against keratin 8 which is not restricted to MCs only. The results reported by McKenna Boot et al. [15] using an anti-NSE antibody were very similar to ours, in that they found numerous MCs in fetal skin (with a peak during the 18th week of gestation), but only a few in normal adult skins. The three markers used in our study, keratin 20, chromogranin A, and NSE, were chosen because of their known reactivity in normal MCs and in Merkel cell tumour [3, 7, 11, 19, 20, 23].

The immunohistochemical results obtained with three different antibodies were not quantitatively similar. NSE was more sensitive for the identification of MCs in nor-

mal skin, whereas keratin 20 was more frequently positive in the MCs of trichoblastomas. There are several possible explanations for these findings. It may simply be due to a regional variation in MCs density, or it may reflect various stages of maturation of these cells, a hypothesis first proposed by Moll et al. [20]. Consideration should also be given to the possibility that other endocrine cell types exist, in addition to MCs. The latter is suggested by the fact that cells with neuroendocrine features sometimes found in basal cell carcinomas [5, 6] and the chromogranin-positive cells of a previously reported case of trabecular carcinoid of the skin [4] were negative for keratin 20. At present it is not possible to exclude the possibility that the major positivity of NSE is consequent on occasional immunoreactive melanocytes.

The presence of numerous MCs in NSJ, in NSJ with a microscopic trichoblastoma, and in trichoblastomas unassociated with NSJ indicates that our results parallel those of Schulz and Hartschuh [21]. Although the latter authors originally explained the presence of MCs in trichoblastoma arising from NSJ on the basis of hyperplasia, they later favoured the divergent differentiation of neoplastic elements as an explanation for MCs in a larger series of trichoblastoma [22]. Whether this is the correct interpretation or whether MCs result from colonization of epidermal-derived elements (in a manner analogous to the colonization by melanocytes of breast carcinoma [2, 14] or basal cell carcinoma [12]) remains to be determined. However, the fact that the MCs present in trichoblastomas are indistinguishable in appearance and distribution from those seen in normal fetal hair germ structures suggests that they are the result of a differentiation in a tumour that recapitules skin embryogenesis. In this regard, it is of interest that no cases of basal cell carcinoma (including those in the present series) or of squamous cell carcinoma of the skin have been described that have contained MCs. The reason may well be that squamous cell carcinoma is a more "adult" tumour, and more committed towards keratinocytic differentiation, whereas basal cell carcinoma may be located at the other end of the spectrum, that is to say too primitive to exhibit demonstrable hair follicle (including MC) differentiation.

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