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Neural cell adhesion molecule immunoreactivity in Merkel cells and Merkel cell tumours

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Abstract We have analysed the expression of the neural cell adhesion molecule (NCAM) in normal Merkel cells of pig and human skin, and in nine neuroendocrine carcinomas of the skin (Merkel cell carcinomas). NCAM immunoreactivity was observed in virtually all Merkel cells, both in epidermis and vibrissae of pig snout skin and in human epidermis. Immunostaining surrounded the entire surface of Merkel cells and was not restricted to the contact areas between Merkel cells and nerve terminals. All Merkel cell carcinomas studied were also positive for NCAM. The immunostaining pattern of the tumour cells was similar to that observed in normal Merkel cells; the immunoreactivity was confined to the cell membranes. These results suggest that NCAM may be used as an immunohistochemical marker for both Merkel cells and Merkel cell tumours.

Key words NCAM · Merkel · Neuroendocrine · Carcinoma · Skin

Introduction

Merkel cells are neuroendocrine cells localized in the skin and oral mucosa. Immunohistochemical studies have shown that Merkel cells express not only different peptides: met-enkephalin [15, 40], vasoactive intestinal polypeptide [10, 16], bombesin [11], calcitonin gene-related peptide [2, 8, 10, 13], substance P [10, 41], peptide histidine isoleucine [10, 13], and pancreastatin [14], but also serotonin [6, 7, 43].

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Neural cell adhesion molecule (NCAM) is the first isolated and most studied cell adhesion molecule, a family of membrane associated glycoproteins which mediate cell-cell adhesion. NCAM acts in a calcium independent manner and exists in three main isoforms of approximately 120, 140 and 180 kDa. Although is was initially identified in nervous tissue [3, 35], hence its name, further studies have demonstrated the presence of NCAM in different cell types including sensory [27, 34, 38, 39, 42] and endocrine or neuroendocrine cells [12, 17, 19, 20, 21].

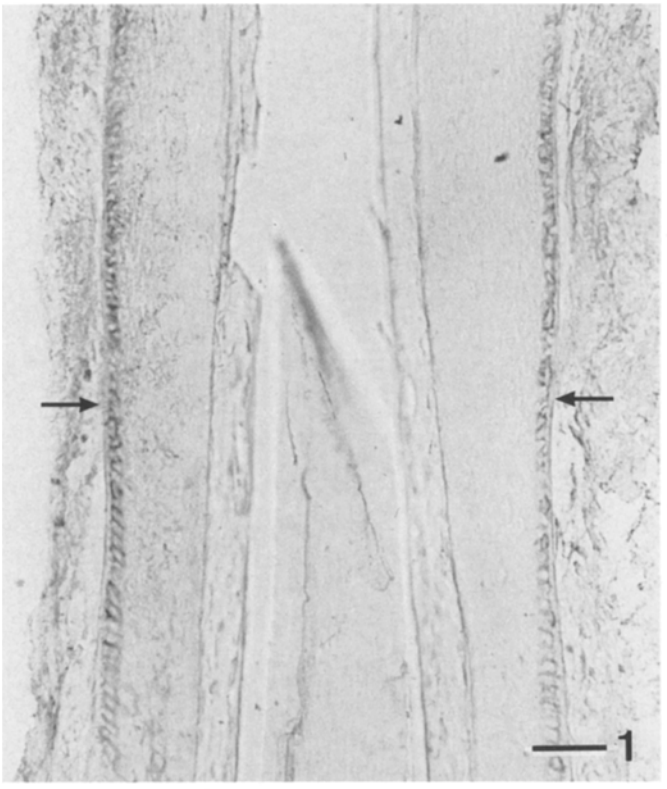
The aim of this work was to investigate the presence of NCAM in neuroendocrine (Merkel) cells of pig and human skin and in Merkel cell carcinomas.

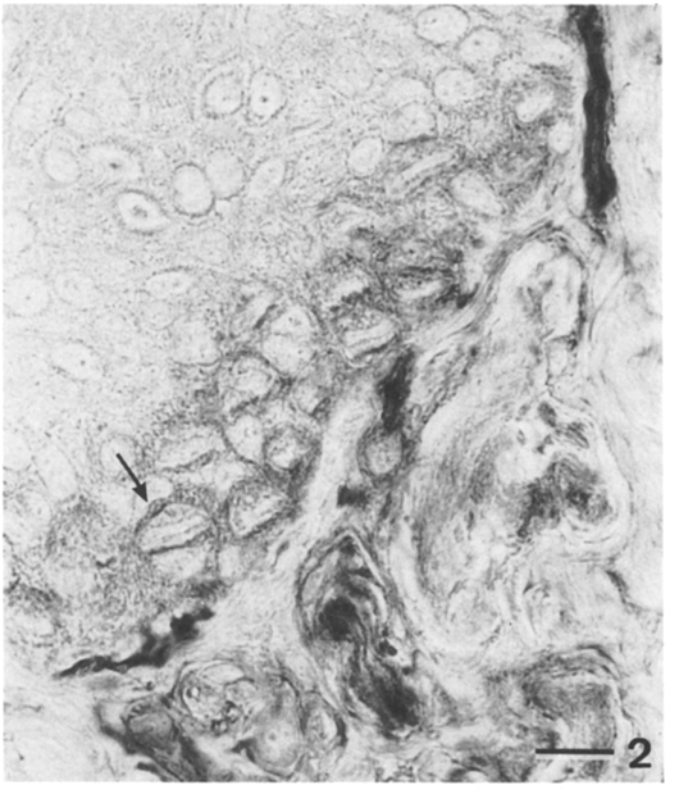
Materials and methods

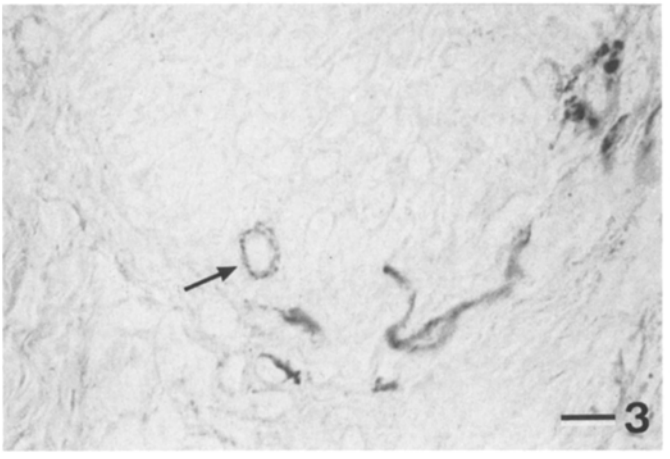
Specimens of pig snouts (four; obtained from the local slaughterhouse), human fingertips (four; obtained from surgical amputations), and Merkel cell carcinomas (nine) were studied. The localization of Merkel cell carcinomas was face (eight cases) ad leg (one case). Five patients were male and four female, and the patients' ages ranged from 51 to 78 years.

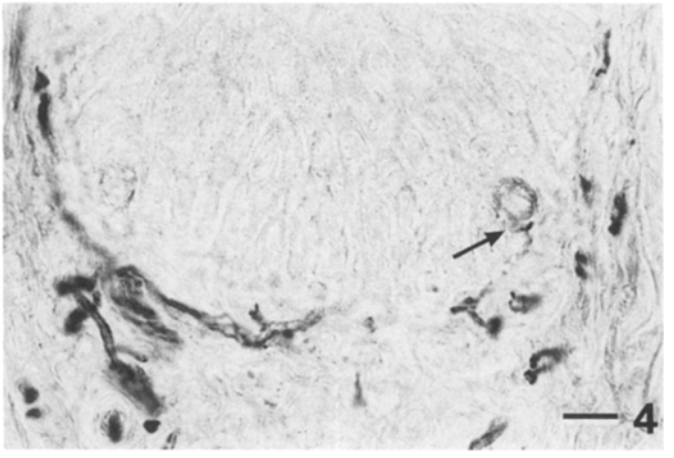
For immunohistochemistry, the avidin-biotin-peroxidase complex procedure was employed. Samples were fixed in 10% buffered formalin for 24 h, dehydrated and embedded in paraffin. Sections 5 μm thick were dewaxed, rehydrated and incubated in VC 1.1 monoclonal antibody (Sigma, St Louis, Mo., USA), at a dilution of 1:200 (for 1 h at room temperature). This antibody localizes the 140 and 180 kDa transmembrane isoforms of NCAM. Additional pig snout and human fingertip samples were embedded in optimal cutting temperature medium, frozen in liquid nitrogen, and cut at −20° C in a 2800 E Frigocut cryostat (Reichert-Jung, Heidelberg, Germany). Frozen sections 7–10 μm thick were fixed in acetone for 10 min (at room temperature), and incubated in T 199 (NKH1 – CD56) monoclonal antibody (Dako, Glostrup, Denmark), at a dilution of 1:20 (overnight at 4° C). This antibody only labels frozen sections and recognizes the 140 kDa isoform of NCAM.

After incubation with primary antibodies, the sections were washed with phosphate-buffered saline (PBS; 0.01 M phosphate buffer pH 7.4 containing 0.15 M sodium chloride) and consecutively incubated in: a) biotinylated horse anti-mouse immunoglobulins (Vector, Burlingame, Calif., USA) at a dilution of 1:100, for 30 min; b) 3% hydrogen peroxide, to block endogenous peroxi-









dase activity, for 10 min (only for paraffin sections); c) avidin-biotin-peroxidase complex (Vectastain Elite kit, Vector), prepared according to the protocol provided by the manufacturer, for 30 min; d) 0.06% (w/v) solution of 3,3' diaminobenzidine-tetrahydrochloride (Sigma) with 0.003% (v/v) hydrogen peroxide, for 10 min. Between steps the sections were washed twice for 5 min with PBS and after step d, with distilled water. All dilutions were made in PBS. No counterstaining was done.

Controls were performed by replacing the primary antibody by either normal mouse serum or PBS, or by omitting any essential step of the reaction. In neither case was immunoreactivity seen.

Results

The results obtained with VC 1.1 and T 199 antibodies to NCAM were similar. In pig snout skin, NCAM immunoreactivity was observed in sinus hair follicles (vibris-

Fig. 1 Sinus hair follicle of pig snout – T 199 antibody to neural cell adhesion molecule (NCAM). NCAM positive Merkel cells are lined in the external root sheath (*arrows*). The immunoreactivity completely outlines the Merkel cells and the intensity of immunostaining varies between different cells. ×250, *bar*=40 μm

Fig. 2 Pig snout epidermis – VC 1.1 antibody to NCAM. A cluster of Merkel cells positive for NCAM is situated at the base of a rete ridge. Immunoreactivity is seen on the entire surface of Merkel cells (*arrow*). Intense positivity is also found in dermal nerve fibres. ×1000, *bar*=10 μm

Fig. 3 Human skin – T199 antibody to NCAM. An isolated Merkel cell immunoreactive for NCAM is situated at the base of an epidermal ridge (*arrow*). Immunostaining shows in this cell a dot-like appearance. Some dermal nerve fibres close to the Merkel cell are also positive. ×700, *bar*=10 μm

Fig. 4 Human skin – VC 1.1 antibody to NCAM. One Merkel cell immunoreactive for NCAM is associated with an epidermal nerve terminal also immunopositive (*arrow*). ×700, *bar*=10 μm

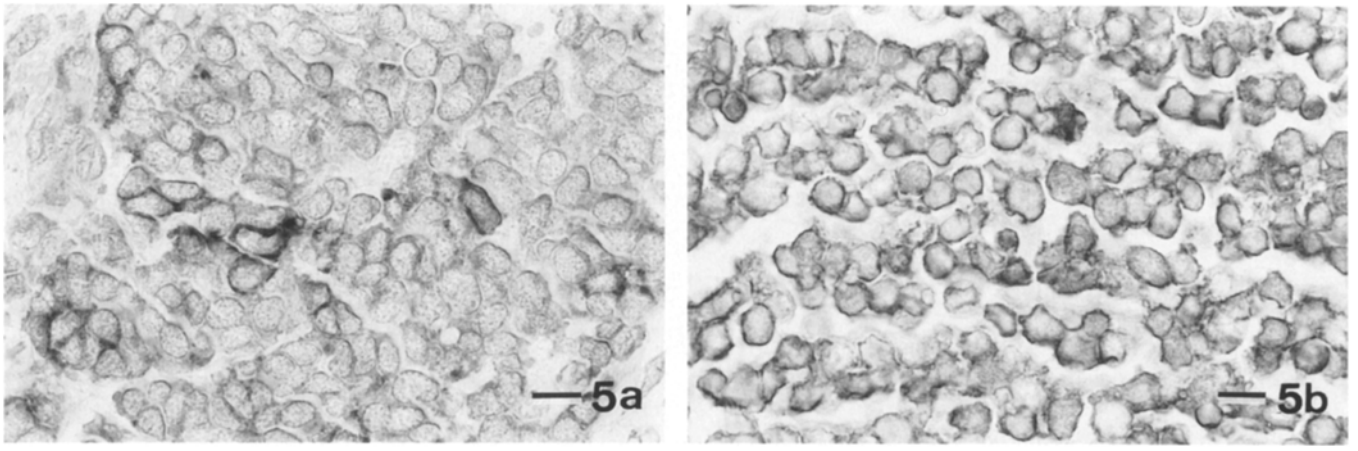


Fig. 5a, b Neuroendocrine carcinomas of the skin – VC 1.1 antibody to NCAM. In some cases only occasional cells show surface immunostaining for NCAM (**a**), but more frequently a diffuse cell membrane immunoreactivity was found (**b**). ×600, *bar*=10 μm

sae) and epidermis. In the vibrissae, the immunostaining was found in the external root sheath where the Merkel cells are localized perpendicularly to the glassy membrane (Fig. 1). In the epidermis, clusters of immunopositive Merkel cells were situated at the base of rete ridges parallel to the basement membrane (Fig. 2). In both localizations virtually all Merkel cells were positive, but differences in the staining intensity were found between them. NCAM immunoreactivity was localized on the surface of the Merkel cells and a cytoplasmic immunoreactivity was never observed. The immunostaining for NCAM completely outlines the Merkel cells and was not restricted to the side where these cells contact with the nerve terminal. As was expected, immunoreactivity was also seen in the nerve fibres (Fig. 2).

In human fingertips, NCAM immunoreactivity was found in isolated round or ovoid Merkel cells situated at the base of epidermal cones (Figs. 3, 4). The immunostaining pattern was identical to that described in the pig Merkel cells, that is to say, the immunoreaction product was seen on the entire surface of Merkel cells. Immunoreactivity was also found in nerve fibres of the superficial dermis, as well as in the nerve terminals associated with Merkel cells (Fig. 4).

All Merkel cell carcinomas studied involved the dermis and subcutis, and no connection with the epidermis was observed. The diagnosis was confirmed by immunohistochemistry and electron microscopy. The nine Merkel cell carcinomas showed NCAM immunoreactivity. As a rule, the majority of tumour cells were positive. However, some variability in the number of immunoreactive cells was found not only between different cases, but also between different fields of the same tumour. As in normal Merkel cells, a diffuse cell membrane staining was always found (Fig. 5).

Discussion

In the skin, NCAM has previously been described in feathers and hairs [4], Pacinian corpuscles [32], and sweat gland ducts [9], but to the best of our knowledge the current study represents the first report of the presence of NCAM in Merkel cells. Merkel cells were immunostained by both anti-NCAM antibodies used in this study. As was expected, the immunoreactivity was localized on the surface of Merkel cells. The absence of a cytoplasmic immunoreactivity for NCAM was expected because it has been shown that the synthesis and transport of NCAM through the Golgi apparatus to the cell membrane are rapid events [22].

The finding of NCAM expression in Merkel cells is not surprising due to the close relationship between neuroendocrine cells and neurons. Moreover, NCAM was previously described in other receptor cells like olfactory cells [27, 39], cochlear hair cells [34, 39, 42] and taste bud cells [29, 31, 36, 38]. As was shown in the receptor cells, immunostaining for NCAM outlined the entire surface of the Merkel cells and was not restricted to that pole of the cells facing the nerve terminal. It has been proposed that in taste buds NCAM might play a role in the growth of gustatory axons toward their target epithelial cells and in recognition between nerve fibres and mature taste receptor cells [36]. The diffuse distribution of NCAM on the surface of these cells was explained by its rapid turnover which implies that new synaptic connections are being made continuously [36, 38]. However, this postulate is not applicable to Merkel cells with a very low proliferation rate [26]. However, since the immunostaining was not restricted to synaptic areas of the receptor cells, other authors postulate that it cannot be argued that NCAM participates in synaptic formation [39].

NCAM has also been described in multiple endocrine and neuroendocrine cells including pituitary cells, adrenal cells, pancreatic islet cells, thyroid C cells, testicular Leydig cells, ovarian granulosa cells and pulmonary neuroendocrine cells [5, 12, 17, 19, 20, 21, 23, 24, 28, 30, 33, 37]. Since most of these cells lack contacts with nerve fibres, NCAM cannot obviously act in nerve-cell

recognition in these cases. The only proposed physiological role of NCAM in the endocrine and neuroendocrine systems is to stabilize the cells into aggregates [21, 24]. This function could be applicable to the Merkel cells forming clusters, but not to the isolated Merkel cells that, as we demonstrated here, also express NCAM. In these isolated cells the homophilic binding mechanism of NCAM cannot be supported and further studies are needed to clarify its role.

NCAM seems to be not only a general marker for neuroendocrine cells, but also for neuroendocrine tumours [1, 9, 17, 18, 19, 20, 28]. In fact, three cases of Merkel cell tumour were previously reported to be positive for NCAM [9, 17]. It has been proposed that antibodies against NCAM can be used as broad-spectrum neuroendocrine markers [17, 20], although we must bear in mind that clearly non-neuroendocrine tissues such as nephroblastomas and regenerating muscle also express NCAM [25, 44]. We suggest the inclusion of NCAM in the routine immunohistochemical battery for the diagnosis of neuroendocrine carcinomas of the skin.

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