Virchows Arch [Pathol Anat] (1985) 406:339–350

Virchows Archiv A

The intermediate filament cytoskeleton of cutaneous neuroendocrine carcinoma (Merkel cell tumour)

Immunohistochemical and biochemical analyses*

H. Höfler[1], H. Kerl[2], E. Lackinger[1], G. Helleis[1], and H. Denk[1]

Institute of Pathology[1], and Department of Dermatology[2], University of Graz, School of Medicine, Auenbruggerplatz 25, A-8036 Graz, Austratia

Summary. The presence and distribution of cytokeratins, neurofilament proteins, vimentin and glial fibrillary acidic protein were studied in 10 cutaneous neuroendocrine carcinomas (CNEC) by immunohistochemical techniques, using specific antibodies. In all cases tumour cells were specifically stained with antibodies to mouse liver cytokeratin component D in paraffin-embedded formalin-fixed and frozen sections. Moreover, one- and two-dimensional SDS-polyacrylamide gel electrophoresis of high salt/detergent resistant cytoskeletal residues from tumour material, isolated by microdissection from frozen sections, revealed the presence of cytokeratin components 8 and 18 which are characteristic constitutents of cytokeratin filaments of simple epithalia. Neurofilament proteins were detected by immunocytochemistry in tumour cells from 2 patients, from whom frozen material was available, and their presence was also positively identified in cytoskeletal residues by immunoblotting using specific antibodies. Glial fibrillary acidic protein and vimentin could not be demonstrated in tumour cells. Our studies did not confirm the suggested origin of CNEC from epidermal Merkel cells.

Key words: Cutaneous neuroendocrine carcinoma – Immunocytochemistry – Gel electrophoresis – Cytoskeleton – Intermediate filaments

Introduction

Cutaneous neuroendocrine carcinomas (CNEC) are neoplasms first described in 1972 by Toker (1972) as trabecular carcinomas. Clinically, bluish-red nodules are observed and the cheek of elderly women is the most frequent single location. Local recurrences after surgery and lymph node metastases are common. The tumour cells are small and uniform in size and

* This work was supported in part by Fonds zur Förderung der wissenschaftlichen Forschung, Grant P4708 to H.D.

Offprint requests to: H. Höfler at the above address

shape with a thin rim of cytoplasm, well defined nuclei, small nucleoli, and finely dispersed chromatin. A few cases with squamous differentiation have been described (Sidhu et al. 1980; Frigerio et al. 1983; Gomez et al. 1983; Fetissof et al. 1983). On the basis of histomorphology the possibility that CNEC represent a heterogenous group of neoplasms cannot be excluded. Electronmicroscopic characteristics are membrane-bound dense-core neuroendocrine granules and intermediate-sized filaments in perinuclear location (Sibley et al. 1980; Pilotti et al. 1982; Hübner et al. 1983; Cremer and Totovic 1983; Warner et al. 1983). The histogenesis of CNEC is unknown. On the basis of ultrastructural similarities a relationship with Merkel cells of the epidermis has been suggested (see Warner et al. 1983, for review).

Using immunohistochemical criteria a more precise definition of CNEC is possible. Several studies have demonstrated the presence of neuron-specific enolase (Gloor et al. 1982; Wick et al. 1983; Gu et al. 1983; Nakashima et al. 1983; Kirkham and Isaacson 1983) and neurofilaments (Mietinen et al. 1983; Viale et al. 1983) suggesting neuroendocrine properties of neoplastic cells in CNEC. There are also reports of CNEC associated with production of ACTH, calcitonin and other peptide hormones (Johannessen and Gould 1980; Iwasaki et al. 1981; Solcia et al. 1981; Gould et al. 1984). In addition, recent data showed that CNEC are also related to epithelial differentiation, because some authors demonstrated cytokeratin (Ortonne et al. 1983) or coexpression of cytokeratin filaments and neurofilaments in tumour cells (Kerl et al. 1984; Merot et al. 1984; Ruiter et al. 1984; Virtanen et al. 1984; Höfler et al. 1984b).

In the present study further immunohistochemical and biochemical analyses were performed to characterize the major cytoskeletal structures of CNEC and thus to contribute to our knowledge regarding histogenesis and diagnosis of these tumors.

Material and methods

Tissues. 10 CNEC, identified by light- and electronmicroscopy, were investigated. Tissues were fixed in 10% buffered formaldehyde solution. Material from 2 patients was frozen immediately after surgery in isopentane cooled in liquid nitrogen for frozen sectioning.

Immunohistochemistry. For the immunocytochemical staining of antigens the PAP-technique (Sternberger 1979) was used. With monoclonal antibodies against neurofilament proteins as first antibody layer the biotin-avidin-peroxidase method (ABC-Kit, Vector, USA) was applied. All reagents were diluted with phosphate-buffered saline (PBS, 0.5 M). Peroxidase activity was visualized with 3′,3′-diamino-benzidine-tetrahydrochloride (0.05% w/v) and hydrogen peroxide (0.01% w/v) in Tris-HCl buffer (0.05 M, pH 7.2). For the immunocytochemical demonstration of cytokeratin the paraffin sections were pretreated with 0.1% pronase (Type VII, Serva, Heidelberg, FRG) for 1 h at 37° C according to Denk et al. (1977). For indirect immunofluorescence microscopy cryostat sections were air-dried for 10 min, fixed in −20° C acetone for 10 min and subsequently incubated with the primary and secondary antibodies (for details see Denk et al. 1981). Double label immunofluorescence microscopy was performed by sequential application of antibodies to cytokeratin and neurofilament proteins from different species and FITC- and TRITC-coupled secondary antibodies. The following antibody combination was used: guinea pig antibodies to mouse liver cytokeratin D (visualized by TRITC-coupled goat antibodies to guinea pig IgG; E-Y-Labs, San Mateo, CA) and mouse antibodies to

Table 1.

Antiserum to (species)	Dilution[a]	Source/References
(gp) mouse liver cytokeratin D (CK/D, 49 K)	1:3,000 1:100[b]	Denk et al. (1981)
(gp) human epidermal prekeratin I (67 K)	1:200 1:80[b]	Denk et al. (1981)
(gp) human epidermal prekeratin II (58 K)	1:1,000 1:40[b]	Denk et al. (1981)
(gp) human epidermal prekeratin III (53 K)	1:1,000 1:60[b]	Denk et al. (1981)
(ra) human vimentin	1:300 1:15[b]	Denk et al. (1983)
(ra) glial fibrillary acidic protein (GFAP)	undiluted	Dako (Denmark)
(m) human neurofilament protein (NF), (70 K), monoclonal	1:10 1:5[b]	Sanbio (Holland)
(m) human neurofilament protein (NF), (200 and 70 K), monoclonal	1:40 1:2[b]	Virtanen (Finland)
(ra) human neuron-specific enolase (NSE), E.C. 4.2.1.11	1:1,000	Dako (Denmark)

Abbreviations: gp = guinea pig; ra = rabbit; m = mouse

[a] peroxidase-antiperoxidase reaction except
[b] indirect immunofluorescence microscopy

human neurofilament protein (kindly provided by Dr. I. Virtanen; visualized by FITC-coupled goat antibodies to mouse IgG and IgM; TAGO, Burlingame, CA). Controls included preimmune rabbit and guinea pig sera, gamma globulin from sheep and PBS, respectively, instead of primary and secondary antibodies. Antibodies used are summarized in Table 1. Antibodies to different epidermal cytokeratin polypeptides were prepared as described by Denk et al. (1981) and Franke et al. (1981) for those to mouse liver cytokeratin D.

Preparation of cytoskeletal material. Tumour tissue from two CNEC was isolated by microdissection of 25 μm thick frozen (cryostat) sections as described by Moll et al. (1982, 1983, 1984). The material was scraped off the slide, collected in medium A (96 mM NaCl, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 1.5 mM KCl, 10 mM Na_2-EDTA, 0.1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride; pH 6.8) and homogenized in a glass homogenizer equipped with a tephlon pestle. Thereafter, 3 vol of extraction medium I (2.0 M KCl, 200 mM NaCl, 10 mM Tris-HCl, 0.1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride) were added and homogenates were extracted for 30 min at 4° C. The homogenate was then centrifuged for 20 min at 10,000 g. The sediment was resuspended in extraction medium II (1.5 M KCl, 1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl, 0.1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride, pH 7.4; 3× the vol of E I) and extracted for 90 min at 4° C. The extract was centrifuged for 20 min at 10,000 g, the sediment was washed 3 times for 20 min in PBS (pH 7.45) containing 0.1 mM dithiothreitol and 0.4 mM phenylmethylsulfonyl fluoride and centrifuged at 8000 g for 5 min.

Gel electrophoresis. One-dimensional SDS-polyacrylamide gel electrophoresis followed the methodology of Laemmli (1970) as described (Denk et al. 1981). Two-dimensional gel electrophoresis was performed using nonequilibrium pH gradient conditions in the first dimension (O'Farrell et al. 1977) and SDS-electrophoresis in the second dimension (Moll et al. 1983).

Gels were stained with Coomassie blue. In order to positively identify some polypeptides, immunoblots were performed according to Towbin et al. (1979) using antibodies to vimentin from human aorta (Denk et al. 1983) and to neurofilament protein (kindly supplied by Dr. I. Virtanen, Helsinki, Finland; see Table 1). Proteins were identified by the binding of antibodies (e.g., to vimentin) to polypeptides transferred to nitrocellulose paper by blotting. Binding of antibodies was visualized by the peroxidase-antiperoxidase (PAP) technique (Sternberger 1979). The binding of monoclonal antibodies (e.g., to neurofilament proteins) was assessed by horse-radish peroxidase-coupled antibodies to mouse immunoglobulins (Dako, Denmark). Peroxidase activity was visualized by the diamino-benzidine reaction.

Results

Morphology and immunohistochemistry

The histological features of the investigated CNEC were variable. Only 2 neoplasms revealed characteristic sheets of cells forming a trabecular pattern, 3 tumours showed features resembling oat cell carcinoma and in 5 lesions a nodular and diffuse pattern simulating malignant (lymphoblastic) lymphoma was found. Neuron-specific enolase immunoreactivity was detectable in all CNEC (for a detailed description and the investigation of different neuropeptides see Höfler et al. 1984b). Tumour cells did not react with antibodies to S-100 protein, glial fibrillary acidic protein (GFAP) and vimentin. A positive reaction for vimentin was found only in the stroma and vessels of CNEC. In all cases of CNEC, tumour cells were stained

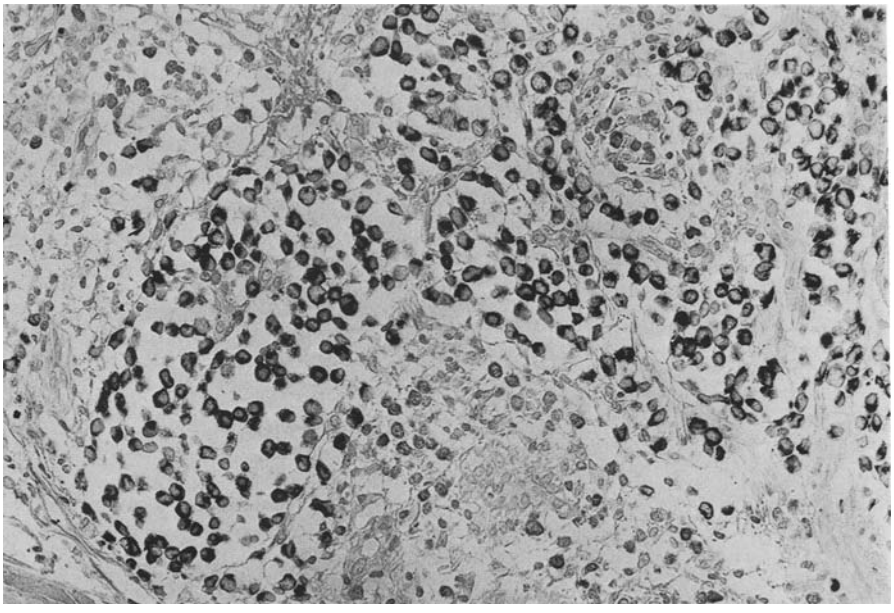

Fig. 1. Cutaneous neuroendocrine carcinoma. Ring-like cytoplasmic staining pattern of tumour cells with anti-cytokeratin D. Formalin fixation, paraffin-embedded material. PAP-technique. ×280

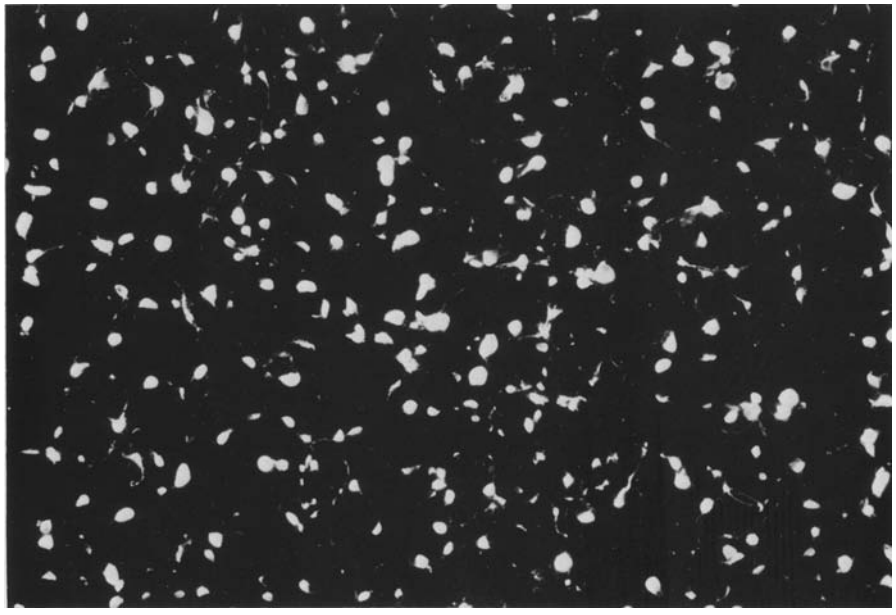

Fig. 2. Cutaneous neuroendocrine carcinoma. Strong fluorescence of paranuclear cytokeratin accumulations in tumor cells. Notice also decoration of slender cytoplasmic extensions. Cryostat section, acetone fixation, indirect immunofluorescence. × 640

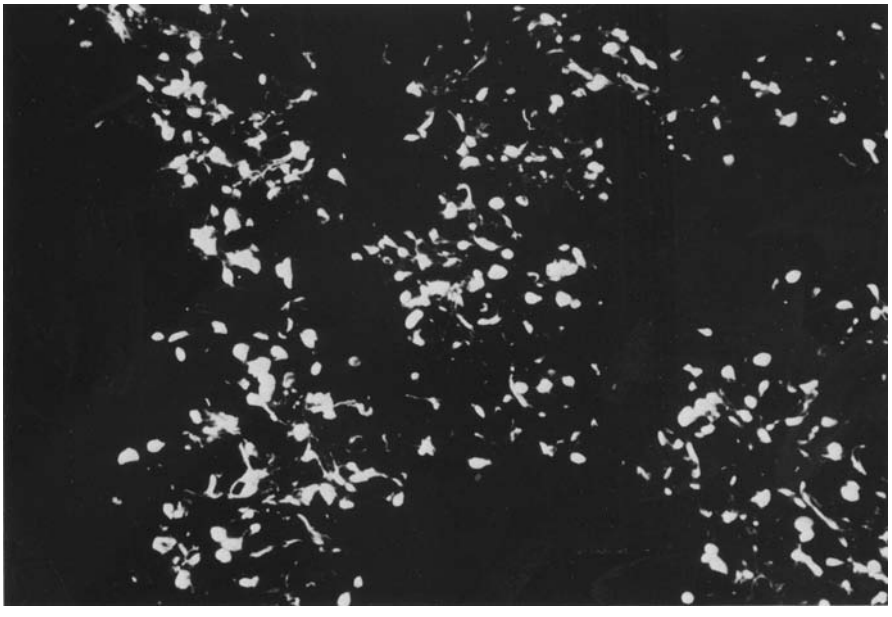

Fig. 3. Cutaneous neuroendocrine carcinoma. Intensive paranuclear fluorescence of tumour cells with antibodies to neurofilament proteins (200 K and 70 K). Staining pattern is similar to that anti-cytokeratin D. Cryostat section, acetone fixation, indirect immunofluorescence. × 400

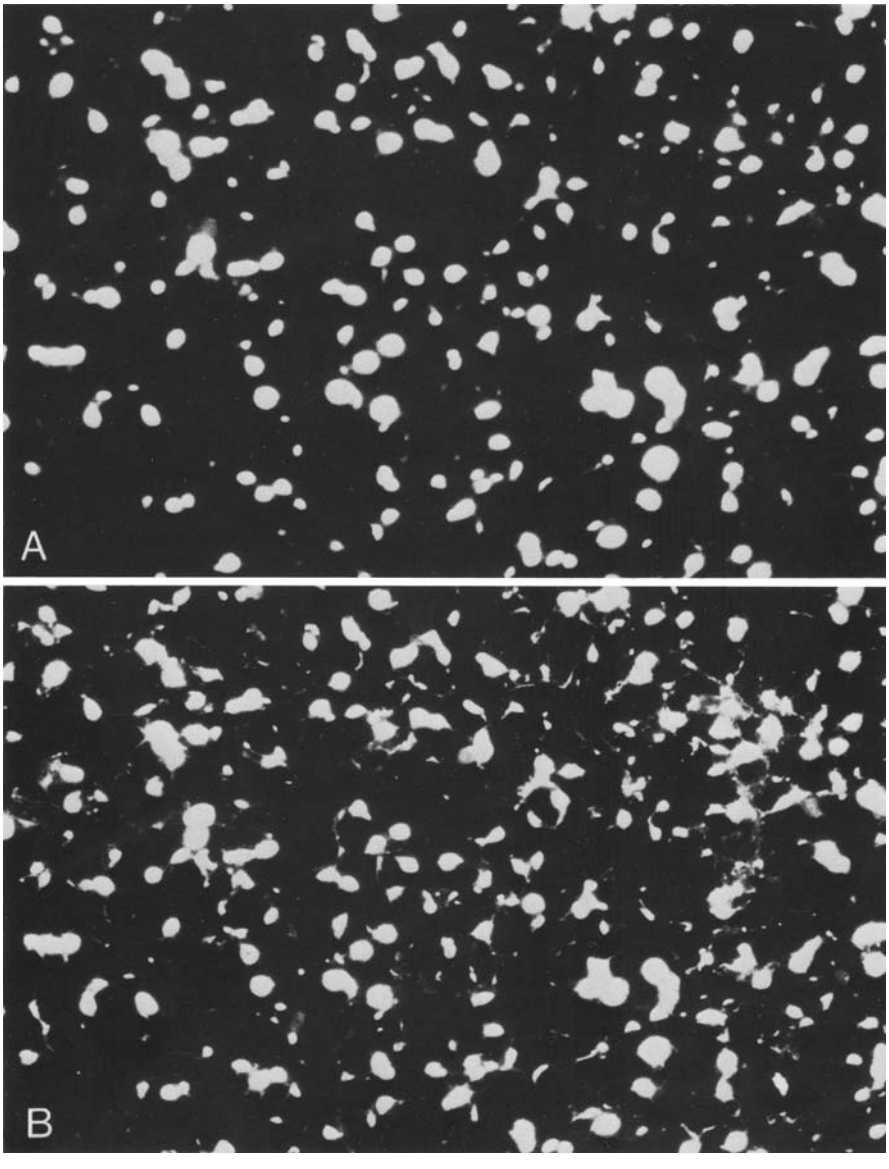

Fig. 4. Cutaneous neuroendocrine carcinoma. Double label immunofluorescence microscopy discloses colocalization of neurofilament proteins (**A**) and cytokeratins (**B**) in tumor cells. Cryostat section, aceton fixation, double label immunofluorescence microscopy (**A**, FITC; **B**, TRITC). ×800

with antibodies to mouse liver cytokeratin D (anti CK/D; for designation see Denk et al. 1981). The cytokeratin-related staining was confined to the rim-like cytoplasm of tumour cells. In some tumour cells immunostaining was concentrated as a paranuclear plaque (Figs. 1, 2). The keratin-specific immunoreactivity correlated well with the ultrastructurally detectable para-

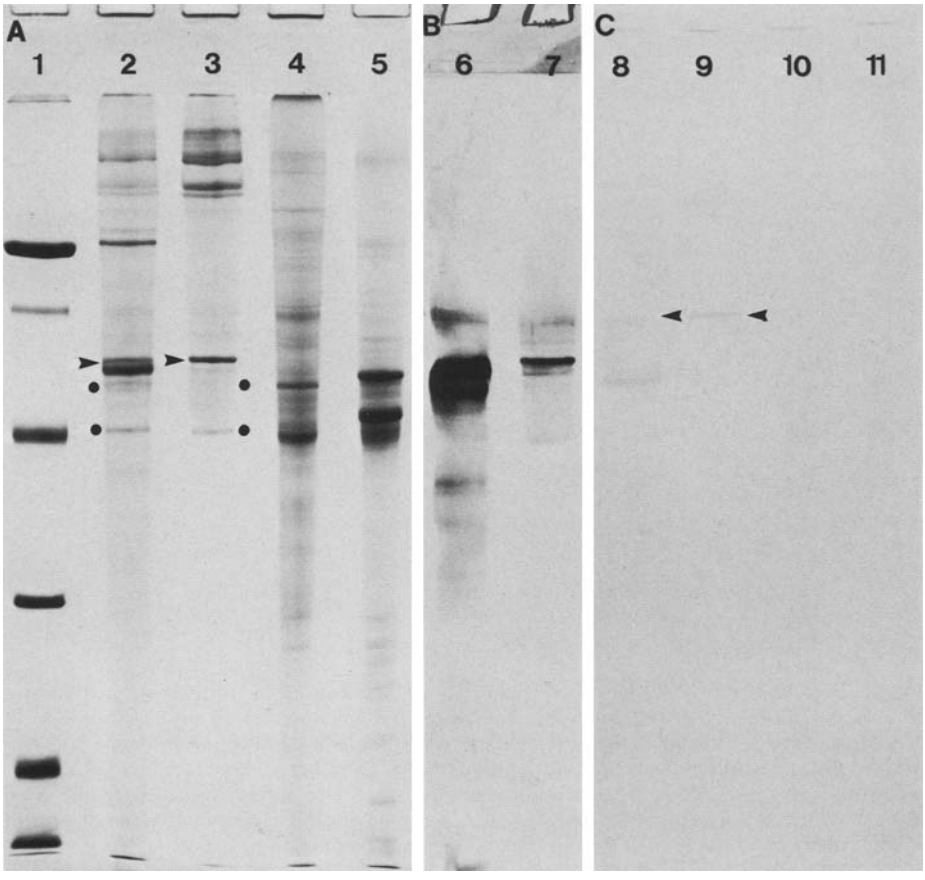

Fig. 5. A One-dimensional SDS-polyacrylamide gel electrophoresis of cytoskeletal (high salt/detergent resistant) residues from two different cutaneous neuroendocrine carcinomas (*lanes 2* and *3*). The tumour tissue was isolated from frozen sections by microdissection prior to the preparation of cytoskeleton enriched in intermediate-sized filaments. Cytokeratin components 8 and 18 (Moll et al. 1982) are indicated by dots (8, upper dot; 18, lower dot in *lanes 2* and *3*). Vimentin and vimentin degradation products are indicated by arrowheads (*lanes 2* and *3*). Cytoskeletal residues from human liver (*lane 4*) with the major polypeptides *8* (*upper band*) and *8* (*lower band*) and from mouse liver (*lane 5*) with the major polypeptides A (*upper band*) and D (*lower band*; for designation see Denk et al. 1981) are included for comparison. Molecular weight markers are shown in *lane 1* (from top to bottom: phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme). **B** Immunoblot of cytoskeletal residues from the two different cutaneous neuroendocrine carcinomas electrophoresed in *lanes 2* (*lane 6*) and *3* (*lane 7*) with antibodies to human vimentin. The bands indicated by arrowheads in *lanes 2* and *3* are positively identified as vimentin and vimentin degradation product (*lower band* in *lane 6* corresponding to *lane 2*). **C** Immunoblot of cytoskeletal residues from the cutaneous neuroendocrine carcinomas shown in lanes 2 and 3 (*lane 8* corresponds to *lane 2*; *lane 9* corresponds to *lane 3*) with antibodies to neurofilament proteins (reactive with the 70 K and the 200 K protein; antibodies kindly supplied by Dr. I. Virtanen). Note positive reaction of the neurofilament antibody with a band with apparent molecular weight between 69 and 70 K (*lanes 8* and *9*; *arrowheads*). In *lane 8* a less distinct band at 57 K is also decorated (degradation product?). The neurofilament antibody does not react with cytoskeletal polypeptides derived from human (*lane 10*) and from mouse (*lane 11*) liver

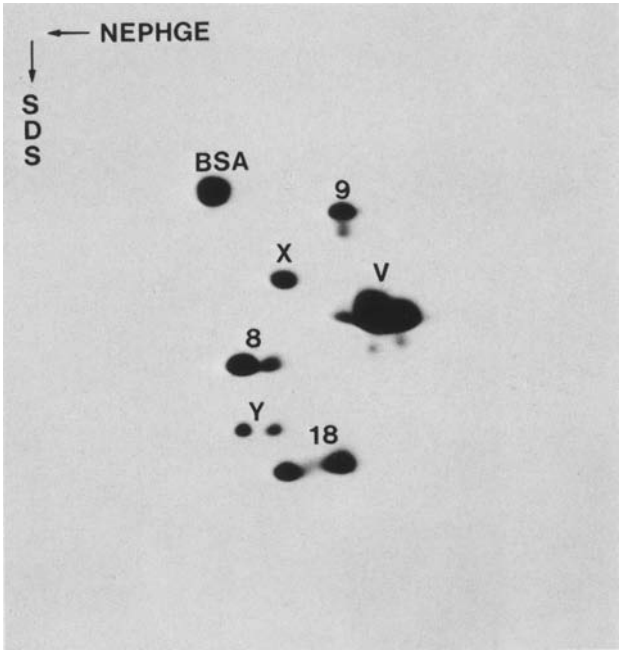

Fig. 6. Two-dimensional gel electrophoretic pattern of cytoskeletal proteins derived from a cutaneous neuroendocrine carcinoma (identical to that electrophoresed in *lane 2* of Fig. 4). NEPHGE, first dimension using non-equilibrium pH gradient electrophoresis (basic polypeptides to the left, acidic polypeptides to the right); *SDS*, direction of second dimension electrophoresis in the presence of SDS. Components 8 and 18 (for further information see Moll et al. 1982) are major cytokeratin polypeptides. A component (*9*) corresponding to component 9 of human epidermis is also present. *X* and *Y* denote unidentified proteins. Vimentin (*V*) is most probably derived from contaminating mesenchymal cells and blood vessels. BSA is included as reference protein

and perinuclear accumulation of intermediate-sized filaments. CNEC with trabecular features showed a more diffuse cytoplasmic staining pattern. All cases tested were positive on paraffin-embedded and frozen sections; the reaction, however, was more pronounced on frozen material. With antibodies specific for epidermal prekeratin polypeptides of different molecular weights (53 K, 58 K, 67 K) negative results were obtained. These antibodies only decorated the epidermis and adnexal structures. A positive staining pattern almost identical to anti CK/D was observed with antibodies directed to neurofilament polypeptides (70 K, 200 K and 70 K), namely cytoplasmic staining with areas of focal concentration (Fig. 3). Sometimes cytoplasmic extensions were decorated. Neurofilament proteins were detectable in frozen sections only. The immunoreactivity was weaker with the antibody to the 70 K neurofilament protein than with that which recognized the 200 K and 70 neurofilament proteins. Double label immunofluorescence microscopy disclosed colocalization of cytokeratin and neurofilament proteins (Fig. 4).

Electrophoretic and immunochemical results

One dimensional SDS-polyacrylamide gel electrophoresis revealed several bands in the molecular weight range of intermediate filament cytoskeletal proteins (Fig. 5; lanes 2 and 3). Polypeptides with molecular weights of 58 K (Fig. 5; lanes 2 and 3) and 57 K (Fig. 5; lane 2) were identified as vimentin and vimentin degradation products by immunoblotting (Fig. 5; lanes 6 and 7). Cytokeratin polypeptides corresponded to components 8 (M_r, 52.5 K) and 18 (M_r, 45 K) according to the catalog of human cytokeratins established by Moll et al. (1982). In two-dimensional gels isoelectric pH values of the major variant were around 6.1 (component 8) and 5.7 (component 18; see Fig. 6). A polypeptide spot corresponding in its electrophoretic coordinates to cytokeratin component 9 (M_r 64.5 K; IEP 5.35) could be detected in small amounts in two-dimensional gels (Fig. 5). Other unidentified spots were also present (X: M_r 59 K, IEP 5.8; Y: M_r 48 K, IEP 6). A polypeptide with electrophoretic coordinates of a neurofilament protein was undetectable in Coomassie blue- and silver-stained gels. Immunoblot experiments on one-dimensional gels, however, revealed a distinct band reactive with antibodies to 200 K and 70 K neurofilament proteins (kindly provided by I. Virtanen, Helsinki, Finland; Fig. 5, lanes 8 and 9).

Discussion

Our immunohistochemical and biochemical studies show that CNEC coexpress cytokeratin filaments and neurofilaments and thereby confirm recent observations (Kerl et al. 1984; Ruiter et al. 1984; Merot et al. 1984; Virtanen et al. 1984; Höfler et al., 1984b). The major cytokeratin polypeptides present in cytoskeleton preparations from CNEC have electrophoretic coordinates corresponding to human cytokeratin components 8 and 18 (for further information see Moll et al. 1982). The polypeptide composition of cytokeratins of CNEC thus conforms to that of nonstratified simple epithelia, e.g. those of the digestive tract and associated glands and organs, including endocrine tissues (Moll et al. 1982; Höfler and Denk 1984). Complex stratified epithelia, on the other hand, express other acidic and basic cytokeratins, mostly with higher molecular weights. The presence of a polypeptide component similar to cytokeratin No. 9, which has so far been identified only in foot sole epidermis (Moll et al. 1982), is as yet unexplained in view of the strict exclusion of epidermis from the microdissected material.

Some investigators were unable to demonstrate cytokeratins in CNEC immunohistochemically (Kirkham and Isaacson 1983; Miettinen et al. 1983). These discrepancies are probably due to the application of cytokeratin antibodies with different specificities. In the present studies antibodies prepared against mouse liver cytokeratin D with broad range of cytokeratin immunoreactivity showed a positive reaction whereas antibodies to higher molecular weight epidermal cytokeratin components yielded negative results. Cytokeratins have now been found in various endocrine tumours in-

cluding gastrointestinal carcinoids and pituitary adenomas (Höfler and Denk 1984; see Höfler et al. 1984a, for further bibliography).

The presence of neurofilaments in CNEC was first emphasized by Miettinen et al. (1983) and Viale et al. (1983). The presence of neurofilament protein (70 K component) has also been confirmed in the present study by immunoblotting using monoclonal antibodies.

The other components of the cytoskeleton investigated in this study, namely vimentin and GFAP, were not detectable in tumour cells of CNEC. Therefore vimentin, found in one- and in two-dimensional gels and positively identified by immunoblotting using vimentin antibodies, most probably results from vimentin filaments derived from stromal cells contaminating the cytoskeletal residues.

CNEC were related to epidermal Merkel cells mainly on the basis of ultrastructural similarities between tumour cells and Merkel cells (Warner et al. 1983). However, CNEC probably have no histogenetic relationship to Merkel cells, and the arguments for this assumption are: (i) most cases of CNEC arise in the dermis and subcutaneous tissue without obvious epidermal involvement or contact with the outer root sheath of hair follicles where Merkel cells are normally located. (ii) CNEC do not contain vasoactive polypeptide (VIP) and met-enkephalin (Höfler et al. 1984b) which are two important markers of Merkel cells (Hartschuh et al. 1983). (iii) In addition, Saurat et al. (1984) demonstrated the lack of neurofilament proteins in normal Merkel cells.

We have presented the coexpression of cytokeratin and neurofilament proteins in CNEC documenting the neural and epithelial properties of the tumour cells. Our results favor the concept that CNEC arise from, or differentiate towards, dermal neuroendocrine cells. There is some similarity between CNEC and gastrointestinal carcinoids which probably originate from subepithelial stromal cells that have migrated downwards into the stroma from the epithelial layer (Höfler and Denk 1984). More decisive answers regarding the histogenesis of CNEC may be expected from systematic studies of the various histomorphological subtypes of CNEC.

Acknowledgments. The authors are grateful to Dr. I. Virtanen (Helsinki) for providing the monoclonal antibodies to human neurofilament protein reactive with 200 K and 70 K proteins. We also thank I. Georgiev for expert photographic work and H. Schleich for the preparation of the manuscript.

References

Cremer H, Totovic V (1983) Der Merkelzell-Tumor der Haut. Licht- und elektronenmikroskopische Untersuchung an fünf Fällen. Pathologe 4:287–293

Denk H, Radaszkiewicz T, Weirich E (1977) Pronase pretreatment of tissue sections enhances sensitivity of the unlabeled antibody (PAP) technique. J Immunol Methods 15:163–167

Denk H, Franke WW, Dragosics B, Zeiler I (1981) Pathology of cytoskeleton of liver cells: demonstration of Mallory bodies (alcoholic hyalin) in murine and human hepatocytes by immunofluorescence microscopy using antibodies to cytokeratin polypeptides from hepatocytes. Hepatology 1:9–20

Denk H, Krepler R, Artlieb U, Gabbiani G, Rungger-Brändle E, Leoncini P, Franke WW (1983) Proteins of intermediate filaments. An immunohistochemical and biochemical approach to the classification of soft tissue tumors. Am J Pathol 110:193–208

Fetissof F, Arbeille-Brassart B, Colombat Ph, Lorette G, Monegier du Sorbier Ch, Jobard P (1983) Tumeur à cellules de Merkel. Ann Pathol 3:285–291

Franke WW, Denk H, Kalt R, Schmid E (1981) Biochemical and immunological identification of cytokeratin proteins present in hepatocytes of mammalian liver tissue. Exp Cell Res 131:299–318

Frigerio B, Capella C, Eusebi V, Tenti P, Azzopardi JG (1983) Merkel cell carcinoma of the skin: the structure and origin of normal Merkel cells. Histopathology 7:229–249

Gloor F, Heitz U, Hofmann E, Höfler H, Maurer R (1982) Das neuroendokrine Merkelzellkarzinom der Haut. Schweiz Med Wochenschr 112:141–148

Gomez LG, DiMaio S, Silva EG, Mackay B (1983) Association between neuroendocrine (Merkel cell) carcinoma and squamous carcinoma of the skin. Am J Surg Pathol 7:171–177

Gould VE, Fodstad O, Memoli VA, Warren WH, Dardy LE, Johannessen JV (1984) Evolution and tumor pathology of the neuroendocrine system. pp 545–580 Falkmer S, Hakanson R, Sundler F (eds) Elsevier, Amsterdam New York Oxford

Gu J, Polak JM, Van Noorden S, Pearse AGE, Marangos PJ, Azzopardi JG (1983) Immunostaining of neuron-specific enolase as a diagnostic tool for Merkel cell tumors. Cancer 52:1039–1043

Hartschuh W, Weihe E, Yanaihara N, Reinecke M (1983) Immunohistochemical localization of vasoactive intestinal polypeptide (VIP) in Merkel cells of various mammals: Evidence for a neuromodulator function of the Merkel cell. J Invest Dermatol 81:361–364

Höfler H, Denk H (1984) Immunocytochemical demonstration of cytokeratin in gastrointestinal carcinoids and their probable precursor cells. Virchows Arch [Pathol Anat] 403:235–240

Höfler H, Denk H, Walter GF (1984a) Immunohistochemical demonstration of cytokeratins in endocrine cells of the human pituitary gland and in pituitary adenomas. Virchows Arch [Pathol Anat] 404:359–368

Höfler H, Kerl H, Rauch H-J, Denk H (1984b) New immunocytochemical observations with diagnostic significance in cutaneous neuroendocrine carcinoma. Am J Dermatopathol 6:525–530

Hübner G, Remberger K, Pielsticker K (1983) Neuroendokrines Karzinom (Merkelzelltumor) der Haut. Pathologe 4:212–218

Iwasaki H, Mitsui T, Kikuchi M, Imai T, Fukushima K (1981) Neuroendocrine carcinoma (trabecular carcinoma) of the skin with ectopic ACTH production. Cancer 48:753–756

Johannessen JV, Gould VE (1980) Neuroendocrine skin carcinoma associated with calcitonin production: A Merkel cell carcinoma? Human Pathol 11 (Suppl):586–589

Kerl H, Höfler H, Rauch H-J, Denk H (1984) New immunocytochemical observations in cutaneous neuroendocrine carcinoma (abstract). J Invest Dermatol 84:541–542

Kirkham N, Isaacson P (1983) Merkel cell carcinoma: a report of three cases with neurone-specific enolase activity. Histopathology 7:251–259

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Merot Y, Margolis RJ, Dahl D, Bhan AK, Suarat JH, Mihm MC Jr (1984) Coexpression of neurofilaments and keratin in cutaneous neuroendocrine (Merkel cell) carcinoma cells (abstract). J Invest Dermatol 82:424

Miettinen M, Lehto V-P, Virtanen I (1983) Neuroendocrine carcinoma of the skin (Merkel cell carcinoma): Ultrastructural and immunohistochemical demonstration of neurofilaments. Ultrastruct Pathol 4:219–225

Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalog of human cytokeratin polypeptides: patterns of expression of specific cytokeratins in normal epithelia, tumors and cultured cells. Cell 31:11–24

Moll R, Krepler R, Franke WW (1983) Complex cytokeratin polypeptide patterns observed in certain human carcinomas. Differentiation 23:256–269

Moll R, Moll I, Franke WW (1984) Differences of expression of cytokeratin polypeptides in various epithelial skin tumors. Arch Dermatol Res 276:349–363

Nakashima N, Goto K, Takeuchi J, Maeda H (1983) Case report of neuroendocrine carcinoma of the skin, histochemical and electron microscopic study. Virchows Arch [Pathol Anat] 401:261–273

O'Farrell PZ, Goodman HM, O'Farrell PH (1977) High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12:1133–1142

Ortonne J-P, Viac J, Fetissof F, Lorette G, Duplay H, Desbas C, Thivolet J (1983) Les cellules du carcinome trabéculaire experiment des antigènes de la kératine (abstract). Ann Dermatol Venereol 110:1045–1046

Pilotti S, Rilke F, Lombardi L (1982) Neuroendocrine (Merkel cell) carcinoma of the skin. Am J Surg Pathol 6:243–254

Ruiter DJ, Van Muyen GNP, Dreff E, Mauw BJ, Warnaar SO (1984) Co-expression of cytokeratin and neurofilament in Merkel cell tumors (abstract). J Cutan Pathol 11:220

Saurat J-H, Merot Y, Didierjean L, Dahl D (1984) Normal rabbit Merkel cells do not express neurofilament proteins. J Invest Dermatol 82:641–642

Sibley RK, Rosai J, Foucar E, Dehner LP, Bosl G (1980) Neuroendocrine (Merkel cell) carcinoma of the skin. A histologic and ultrastructural study of two cases. Am J Surg Pathol 4:211–221

Sidhu GS, Feiner H, Flotte ThJ, Mullins JD, Schaefler K, Schultenover StJ (1980) Merkel cell neoplasms. Histology, electron microscopy, biology, and histogenesis. Am J Dermatopathol 2:101–119

Solcia E, Capella C, Buffa R, Usellini L, Fiocca R (1981) Cytology and biology of gut endocrine tumors. In: Bloom SR, Poalk JM (eds) Got Hormones. Churchill-Livingstone, Edinburgh, pp 541–546

Sternberger LA (1979) The unlabeled antibody peroxidase-antiperoxidase (PAP) method. In: Sternberger LA (ed) Immunocytochemistry (2nd ed). John Wiley, New York, pp 104–169

Toker C (1972) Trabecular carcinoma of the skin. Arch Dermatol 105:107–110

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354

Accepted February 11, 1985