Virchows Archiv A Pathol Anat (1989) 414:199–208

Virchows Archiv A
Pathological Anatomy and Histopathology

Pigmented esthesioneuroblastoma showing dual differentiation following transplantation in nude mice

An immunohistochemical, electron microscopical, and cytogenetic analysis*

A. Llombart-Bosch, C. Carda, A. Peydro-Olaya, R. Noguera, J. Boix, and A. Pellin
Department of Pathology, Hospital Clinico, University of Valencia Medical School, Av Blasco Ibañez 17, Valencia 46010, Spain

Summary. Esthesioneuroblastoma (ESTH) is a neuroepithelial-cell-derived neoplasm of the olfactory mucosa composed of homogeneous small round cells which contain neurosecretory granules. Melanin has been detected in such tumours only occasionally. Here we describe a new case of ESTH with divergent differentiation. The primary neoplasm was found in a 67 year-old female, involving the left nasal and maxillary sinus; she died of cerebral metastasis ten months after diagnosis. Histologically only small round cells were seen, with S-100 and NSE positivity. Electron microscopy revealed neurosecretory granules and filaments, as well as the occasional presence of melanosomes. A nude mice xenograft line has been established, and is presently in its ninth transfer. Two cell types are present: small round-to-spindle shaped cells with neural features, and large epithelial-like ones. Both immunohistochemistry and electron microscopy confirm this dual differentiation, with the presence of membrane-bound dense-core neural secretion, as well as melanosomes of neuroectodermal origin. Additionally, an in vitro cell line has been established. Cytogenetic analysis confirmed the presence of both malignant human melanoma patterns; non-random abnormalities in chromosomes 1 and 6, extra copies of chromosome 7. Duplication of the long arm of chromosome 14, as seen in olfactory neuroblastoma, is also seen.

Key words: Esthesioneuroblastoma – Neurosecretion – Melanosomes

* This work has been supported, in part, with a grant (83/058) from the US-Spain Joint Committee for Scientific and Technological Cooperation (Madrid). This paper was presented, in abbreviated form, at the 72nd Tagung of the Deutsche Gesellschaft für Pathologie, Hannover (24–28 May, 1988)

Offprint requests to: A. Llombart-Bosch

Introduction

Esthesioneuroblastoma (ESTH) is an unusual tumour derived from the neuroepithelial cells of the olfactory mucosa which may extend into the ethmoidal sinus, maxillary antrum, sphenoid sinus, nasopharynx and cribriform plate or base of the skull. Histologically this neoplasm shows a homogeneous population, composed of small round cells, distributed diffusely or in Homer-Wright rosettes with a neuropil-like matrix. Ultrastructurally it shows neurotubules and dense-core membrane-bound neurosecretion.

The presence of melanin-like pigment has been reported in one case by Curtis and Rubinstein (1982) with optical and EM support. This pigmented ESTH correlates with other peripheral neuroectodermal tumours which display associated melanin secretion (pigmented neuroectodermal tumour of infancy, melanotic progonoma, melanotic Schwannoma, pigmented differentiated neuroblastoma).

Here we report a new case of ESTH with dual differentiation showing neurosecretion and melanosomes within the same cellular elements of the tumour. We provide immunohistochemical and EM support for this statement not only for the original neoplasm but also for the nude mice xenografts into which the tumour has been successfully transplanted for nine generations. Moreover, short-term tissue culture of the cells provides a cytogenetic basis for this divergent differentiation with the presence of chromosome markers for both ESTH and melanoma.

Material and methods

A 67 year-old woman suffered from a painful swelling over the maxillary sinus, associated with diplopia and oedema of

Table 1. Immunohistochemical antibodies used in the study, indicating source, methods and dilutions employed

Antibodies against	Source	Method	Dilution
Neuron specific enolase (M gamma)	mouse (Innogenetics)[1]	ABC	1/500
HNK-1 (LEU-7) (M)**	mouse	ABC	1/5
Beta-2 microglobulin (P)	rabbit (Accurate Chem Co)[2]	PAP	1/200
LICR – LON 36 (M)***	mouse	ABC	1/5
Chromogranin (M)	mouse (Hybritech)[3]	ABC	1/200
Synaptophysin (M)	mouse (Boehringer)[4]	ABC	1/50
Protein S-100 (P)	rabbit (DAKO)[5]	PAP	1/200
GFAP (P)	rabbit (DAKO)[5]	PAP	1/500*
Neurofilament PROT 70 kD (M)	mouse (SAMBIO)[6]	ABC	undiluted
Vimentin (M)	mouse (DAKO)[5]	ABC	1/30
Cytokeratin (Type 6,18) (M)	mouse (DAKO)[5]	ABC	1/100

(M) monoclonal antibody
(P) polyclonal antibody

[1] Innogenetics NV, Antwerp, Belgium
[2] Accurate Chem Co, Westbury, New York, USA
[3] Hybritech Europe SA, Liege, Belgium
[4] Boehringer Mannheim GmbH, Mannheim, FRG
[5] Dakopatts AS, Glostrup, Denmark
[6] Sambio, Nistelrode, Holland
* incubation overnight at 4° C

Our kind thanks to Dr. Triche (Bethesda USA)** and Dr. Munro-Neville (Sutton, UK)*** for providing us the indicated immunosera

the left eyelids. This coexisted with frontal headache and nasal ventilatory impairment. X-ray examination revealed a huge infiltrating mass extending throughout the nasal cavity and infiltrating the left maxillary antrum, orbital space and cribiform plate. Distant metastases were absent at diagnosis.

Frozen section study of the tumour was performed with the diagnosis of "malignant small round cell neoplasm" compatible with ESTH. This diagnosis was confirmed by histology, immunohistochemistry and EM. Xenografts from the tumour mass were transferred into nude mice (nu/nu). A representative number of tumour fragments were paraffin-embedded (formalin-fixed) and slides were prepared for light microscopy, staining with haematoxylin-eosin, hematein-erythrosin-safranin (HES), periodic acid-Schiff (PAS), Gomori for reticulin and Best's carmine for glycogen. The Masson-Fontana stain was used for the detection of melanin pigment. The indirect immunohistochemical method was used, following the peroxidase-antiperoxidase (PAP) or Avidin-Biotin complex (ABC) methods, as previously described (Sternberger et al. 1970; Hsu et al. 1981; Burns 1982). We used primary monoclonal antibodies directed against neuron specific enolase (gamma), neurofilaments (70 kd), HNK 1 (leu 7), Lirc Lon-36, chromogranin (1k 2H 10), synaptophysin, vimentin and cytokeratin of low molecular weight. We also evaluated polyclonal rabbit antisera for S-100 protein and glyofibrilar acidic protein. Further details pertaining to all these antibodies are illustrated in Table 1. The paraffin-embedded specimens were dewaxed in absolute ethanol and xylol, then incubated for ten min in methanolic hydrogen peroxide solution (1.5%). They were then rehydrated in graded solutions, distilled water and phosphate-buffered saline (PBS) pH 7.5. Primary antibodies were then applied and the sections were incubated for 18 h at 4 degrees C in moisture chambers. For the PAP method, sheep and rabbit globulin and rabbit peroxidase-antiperoxidase complexes were used, while for the ABC technique biotinilated horse antimouse globulin and ABC were used.

After a sequential application of all these reagents, the sections were immersed in 3,3′ DAB solution (0.25 mg/ml in phosphate buffer) with 0.03% hydrogen peroxide for 10 min; they were rinsed in tap water and counterstained with haematoxylin, dehydrated and coverslipped with Permount. The negative controls were stained with substitution of non-immune mouse ascitic fluid or with rabbit serum for the primary antibodies.

For electron microscopy we fixed one cubic millimeter fragments in phosphate-buffered glutaraldehyde (pH 6.9 at 4 degrees C), washed in Millonig solution and then postfixed in 1% osmium tetroxide. The tissue blocks were then progressively dehydrated in graded solutions of ethanol acetone, immersed in propylene oxide and embedded in EPON. An LKB ultramicrotome was used for cutting sections, which were then double-stained with uranyl acetate and lead citrate (Venable and Goggenshau 1965). Examination of the sections was made with both a JEOL 100B and a ZEISS 10A electron microscope.

Short-term cell cultures were obtained from the heterotransplants into nude mice (5th and 7th passages). Each tumour was rinsed in sterile PBS and chemically dissociated with collagenase II-DNAase. The cells were rinsed twice before culturing in RPMI 1640 containing 20% FBS, 1% Pen-Strep and L-glutamine. Both cultures were harvested for cytogenetic analysis in the first passage (24–48 hrs) by routine methods. After hypotonic treatment with 0.075 M KCL, the cells were fixed in methanol glacial acetic acid (3:1 V/V). Slides were air-dried and treated to obtain trypsin G-banding. Twenty-five metaphase spreads from each tumour sample were analysed for chromosome composition and metaphases were karyo-

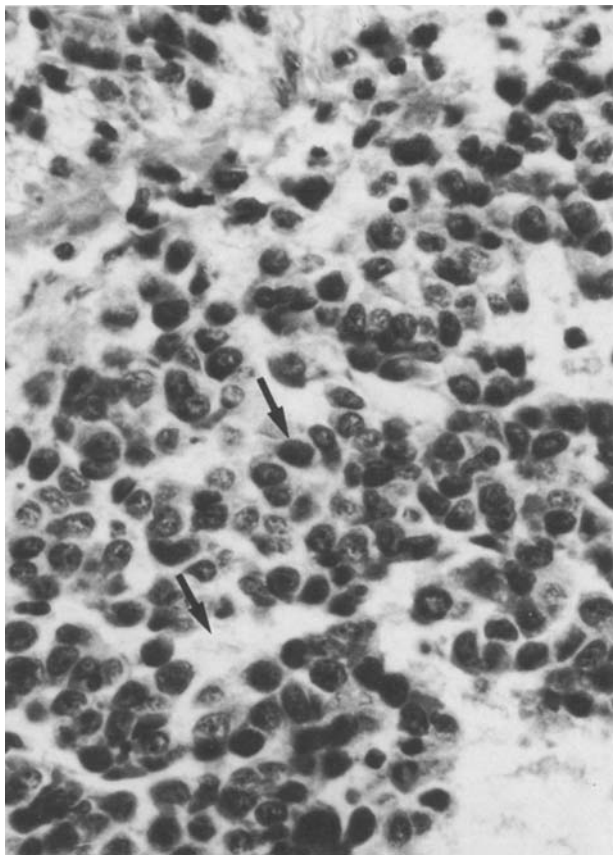


Fig. 1. Histological appearance of the original neoplasm. Presence of abundant small round cells, either isolated or in pseudorosette-like figures. (*Arrows* indicate the presence of rosettes) (HE 300 ×)

typed according to the Paris Conference specifications (ISCN 1978).

Histopathologically, the tumour was made up of a uniform population of small round cells with basophilic nuclei and small, indistinct nucleoli; the cytoplasm was scanty with an occasional neuropil-like appearance. Isolated Homer-Wright rosettes of the neuroblastoma type were seen throughout. Mitotic figures were identified. No mature ganglion cells were present. The stroma was markedly haemorrhagic, showing isolated neoformed vessels. Necrotic foci were abundant but no microcalcification was observed (Fig. 1).

We could not detect any second cell variant within the neoplasm other than the previously mentioned small cell type. Furthermore, no pigment granules of melanotic type were observed. The Masson-Fontana stain for melanin proved negative.

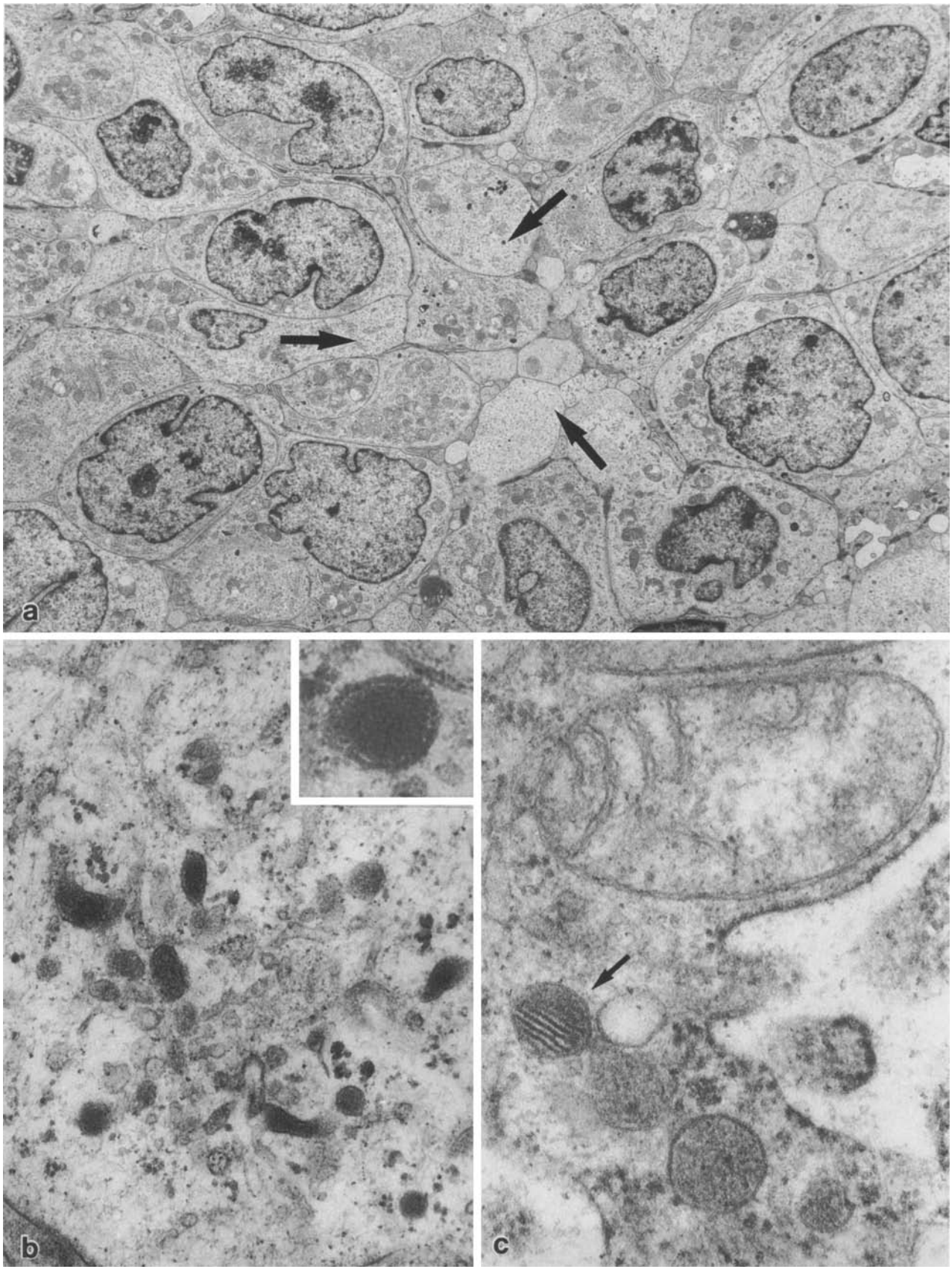
Immunohistochemistry of the neoplasm showed positivity for S-100 protein as well as for NSE in almost all the cells. Additional techniques which proved positive were HNK-1 (leu-7) and E-36. Both these immunosera have shown positivity for neuroectodermal tumours (Llombart-Bosch et al. 1987, 1988). Antibodies to neurofilament protein, chromogranin and cytokeratin of low molecular weight did not react in paraffin-embedded tissue.

At EM level we were able to confirm the neural differentiation of the neoplasm: small round-to-elongated cells and abundant cell processes were closely intermingled. Cells displayed round or irregular nuclei, finely dispersed chromatin and inconspicuous cytoplasm (Fig. 2a). Cytoplasmic organelles were composed mainly of small and large mitochondria, as well as isolated bundles of intermediate filaments. Within the cell processes abundant dense-core membrane-bound granules were found; they were also found within the cytoplasm but in isolated form. Heterogeneity in the size and contour of these granules existed. Small, typical membrane-bound, dense-core granules mimicked those described by EM in neuroblastoma and PNET, being of 60–300 nm in size. The central core was dense and homogeneously stained (Fig. 2b).

A second type of cell granule, not related to lysosomes, was found. These second cell granules were occasionally lacking in membranes while the core showed cross striation periodicity. The size and contour of these granules were several times larger than those previously described (300–800 nm), displaying elliptical, cigar-shaped contours, with features of stage II premelanosomes as well as stages III and IV melanosomes (Fig. 2c). We could not find "neuromelanin pigment" within the cells; ultrastructurally no association existed between this granular material and the lysosomal bodies, as has been described in neuromelanin pigment (Mullins 1980; Curtis and Rubinstein 1982).

Both types of granules, neurosecretory and pigmented, were seen simultaneously within the same cell cytoplasm, a fact that indicates a dual secretory capability for these neoplastic cells.

The original neoplasm was transferred to three nude mice by subcutaneous implant into the back of the animals and successively passed on in nine serial transfers. The number of positive takes was nearly 100% of cases (only one nude mouse out of 27 successively transferred animals was negative). Intervals between tumour passages decreased progressively from 135 days (first passage) to 86.7 days (at present the ninth passage). The neoplasm grew to form confluent nodules, which reached a weight of up to 14.5 grams. From the first passages


a
b
c

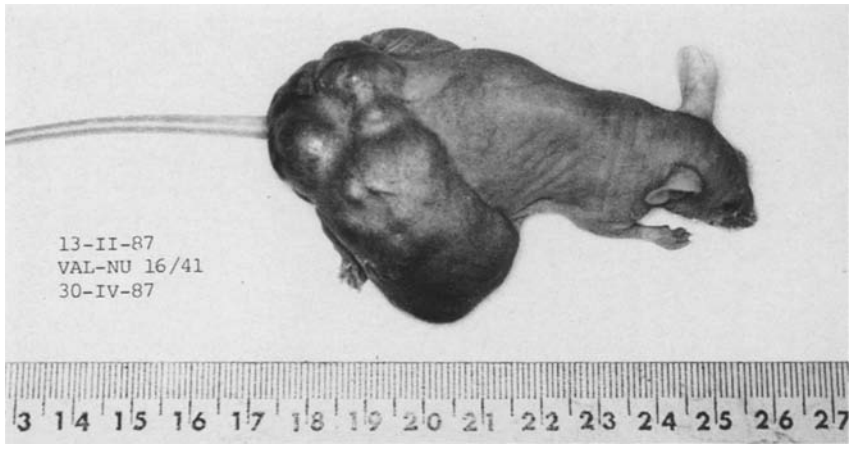


Fig. 3. Nude mouse from xenograft No. 16/41 (5th passage) after 75 days of transplant is shown, with a huge tumour mass on the back

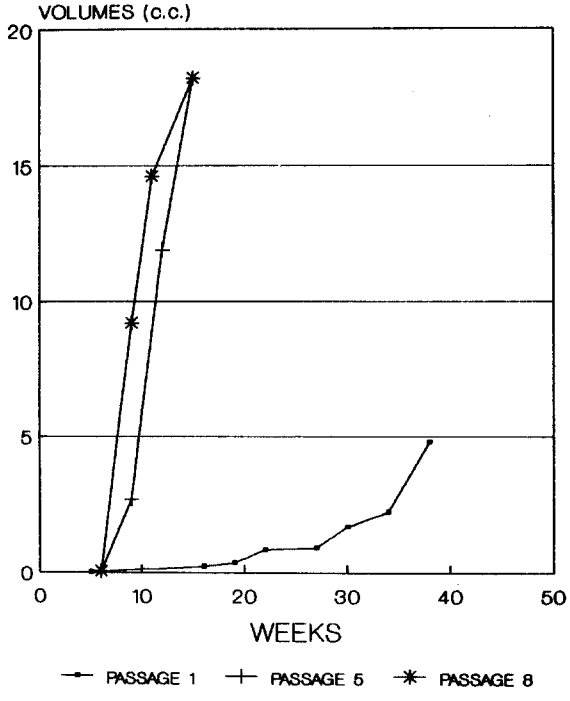


Fig. 3b illustrates the course of the xenografts in the successive tumour transfers with a progressive shortening in the transplantation time from passage No. 1 to No. 8

onwards the neoplasms showed a dark color, being fleshy and haemorrhagic. One nude mouse suffered a lung metastasis, the only case of distant spread (Figs. 3a, b).

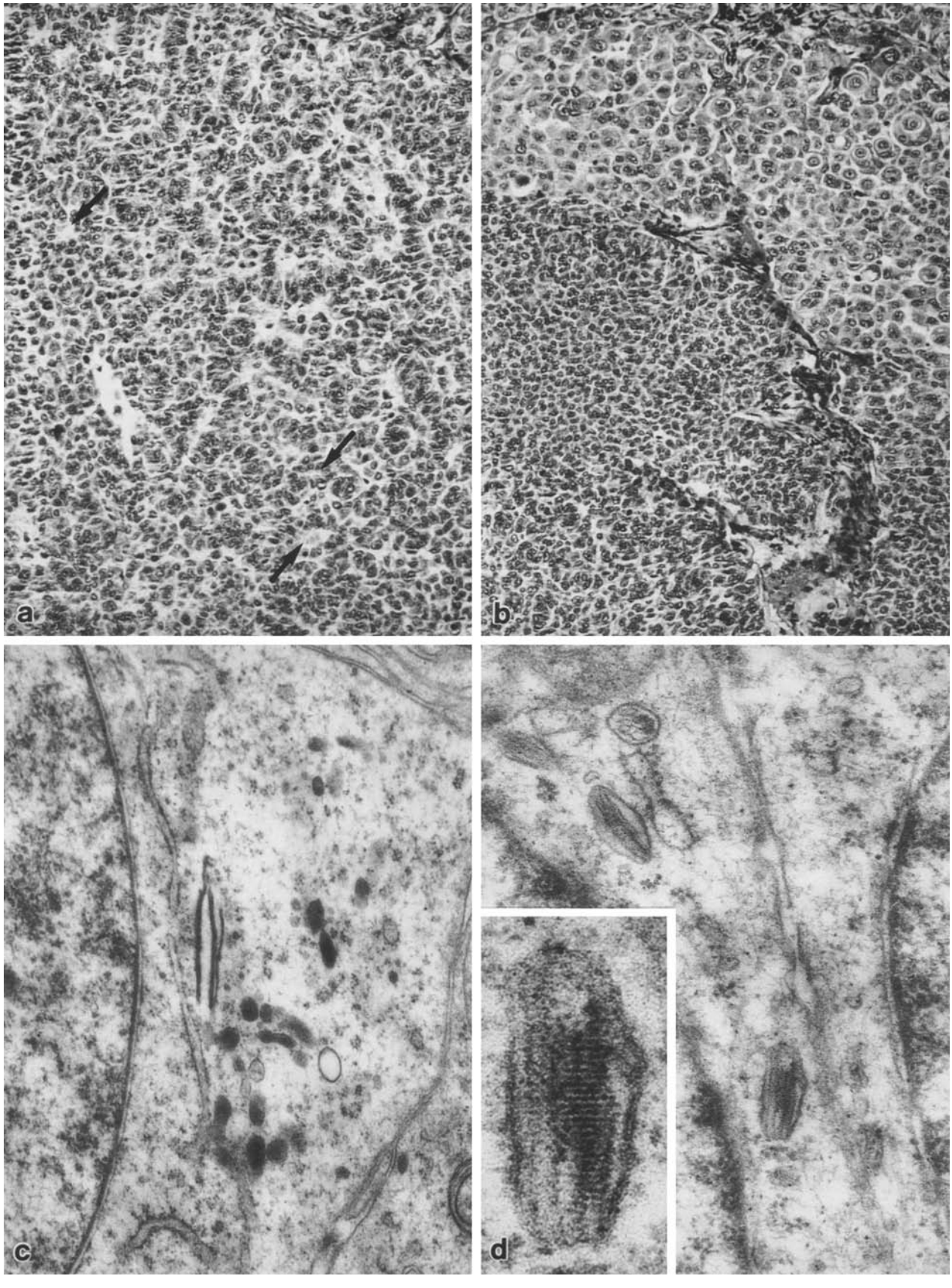
At histology the nude mouse implanted tumour displayed a more heterogeneous pattern than the original neoplasm. At least two cell types could clearly be delineated by HE stain in paraffin-embedded tissue. Small round cells decreased successively in number; they were more elongated and spindle shaped. The nuclear irregularities increased successively. Some of these cells contained melanin, confirmed by the Masson-Fontana technique. A second cell type, less numerous than the former, was also found, displaying an epithelioid pattern with a large polygonally contoured cytoplasm and prominent nuclei with large nucleoli. Most of these cells stored abundant melanin within the cytoplasm (Fig. 4a, b).

Immunohistochemistry of this transferred tumour showed a dual expression of the neural markers. NSE was positive for all cells (small and large epithelioid) while S-100 protein was positive only for the large epithelioid and isolated small, pigmented cells. Vimentin proved positive in all cell types but low-weight cytokeratin resulted negative. Other neural markers, such as HNK-1 and E-36, proved positive in some isolated cells while neurofilament expression resulted negative.

Electron microscopy of several transplanted tumours confirmed previous EM analyses in the original neoplasm with the concomitant expression of both types of granules: neurosecretion and melanin (Fig. 4c, d).

In cell culture the tumour cells grew slowly and attached as monolayers on the fourth day following retrieval. The neuroblast-like cells were distinguishable from fibroblasts by their typical mor-

Fig. 2. Electron microscopic appearance of the original neoplasm. (**a**) Shows a general view of the tumour. Small, undifferentiated cells converge toward a central core with numerous cytoplasmic processes (*Arrows*) (3500×). (**b**) Presence of neurosecretory granules with dense cores and membrane-bound structures (50000×). In the *inset* a higher magnification of one such neurosecretory granule is displayed (120000×). (**c**) Demonstrates the existence of melanosomes within the tumour cells with a striated configuration (*Arrow*) (90000×)


a
b
c
d

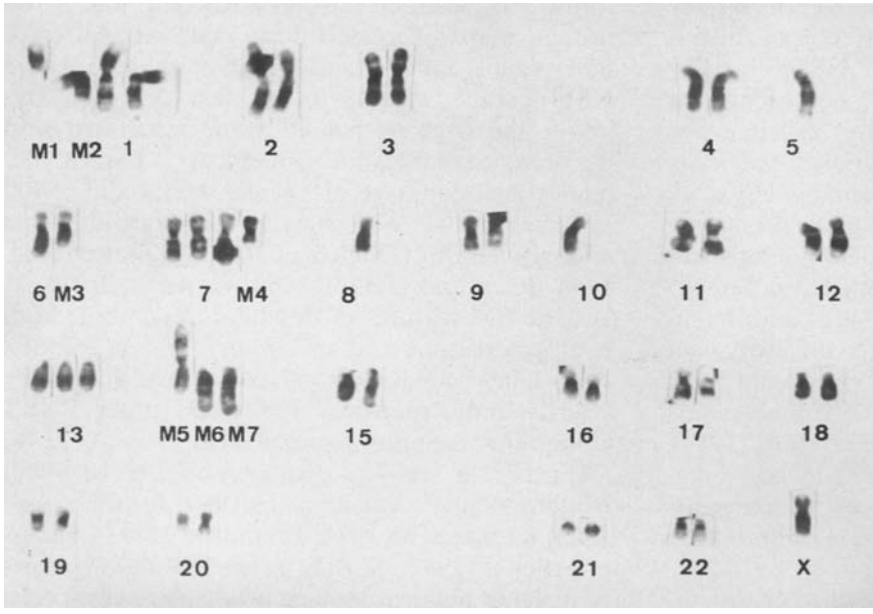


Fig. 5. Karyotype of the transplanted neoplasm into nude mice. Presence of several chromosomal abnormalities and markers (M1–M7). For further details see text

phology (displaying triangular and spindle-shaped forms with large nuclei with single, predominant nucleoli). The chromosome composition of both the 5th and 7th passages was found to be similar in terms of modal number, structure and numerical aberrations. The chromosome number ranged from 45 to 96 with a modal number of 48. Seven consistent marker chromosomes were observed. The origin of these markers was identified as follows: M1 del(1) (11), M2 del(1) (32), M3 del(6) (q21), M4 del(7) (22), M5 t(1,14) (p11, q11), M6 = M7 dup(14) (q24, q32) (Fig. 5).

Discussion

ESTH is an unusual tumour which appears both in children and adults, primarily located in the upper portion of the nasal cavity with regional extension and CNS metastatic capacity, as well as distal colonization in lymph nodes and lung (Kadish et al. 1976; Homzie and Elkow 1980; Silva et al. 1982; Mills and Frierson 1985; Newbill et al. 1985). The neoplasm displays a poor prognosis when this regional extension occurs, as in the case here presented, which, within a 10-month period, the tumour extended locally into the base of the skull and infiltrated the CNS; further lymph nodes and lung metastasis occurred, leading to death of the patient. Local radiotherapy failed to prevent the huge extension of the neoplasm.

The microscopical features of ESTH have been clearly fixed since the first description by Berger et al. in 1924. The tumour appears to be made up of small round-to-elongated cells with Homer-Wright rosettes or a diffuse pattern. Haemorrhages, necrosis and focal microcalcification may occur. Histology provides no prognostic significant data, as stated by Mills and Frierson (1985).

The immunohistochemistry of this neoplasm has not been extensively analysed, but there appears to be no ESTH-specific antigen reliably identifiable in either fresh tissue or paraffin-embedded material. The S-100 protein and NSE are frequently present in the tumour (Choi and Anderson 1985; Axe and Kuhajda 1987; Ng et al. 1988). Other markers, such as neurofilaments and chromogranin may be present in isolated cases (Osborn et al. 1982; Trojanowski et al. 1982, 1984; Vollruth et al. 1984). Micheau et al. (1975) have reported positivity for catecholamine and dopamine beta-hydroxilase, which is an uncommon finding in peripheral neuroectodermal tumours (PNET). Tissue culture reports on two cell lines of ESTH have been published by Cavazzana et al. (1988). Here

Fig. 4. Optical and electron microscopical characteristics of the tumour transferred to nude mice. (**a**) Shows the predominant appearance of the neoplasm: small spindle cells distributed in rosette-like figures (*Arrows*) (HE 100×). (**b**) A second cell type, of large epithelioid appearance, rich in melanin pigment, is seen (HE 100×). In both cell types, but predominantly in the large, neurosecretory granules (**c**) 25000× and abundant melanosomes (**d**) 50000× are found (*Inset* at 200000×)

the immunohistochemistry on isolated cells largely supports the peripheral neural character of this tumour with positive expression of NSE, chromogranin, synaptophysin and neurofilaments (160 KD); a positive finding in this cell line was the presence of cytokeratin. Focal positive staining for cytokeratin has also been seen by Ng et al. (1988) in two olfactory neuroblastomas, presenting primarily as intracranial tumours. Both neoplasms showed prominent features of epithelial differentiation, leading to the initial diagnosis of undifferentiated carcinoma. In addition, mixed olfactory neuroblastoma and carcinoma have been confirmed to have adenocarcinomatous and squamous differentiation ultrastructurally by Miller et al. (1984). None of these cases has displayed melanin pigment. The case discussed here shows positivity for NSE and S-100 protein, but cytokeratin and other neural markers resulted negative.

Several EM analyses have helped to characterize the fine structure of this tumour (Kahn 1974; Mackay et al. 1976; Osamura and Fine 1976; Taxy and Hidvegi 1977; Chaudhry et al. 1979; Silva et al. 1982; Cavazzana et al. 1988). There are membrane-bound neurosecretory granules from 60 to 300 nm in size in the cell perikaryon and in the cell processes. Moreover, longitudinally oriented microtubules and microfibrils have also been identified in the numerous cell processes. All these structures were present in our case.

Nude mice xenotransplants provide a useful tool for the study of cell heterogeneity in human tumours, as the degree of differentiation and histology can be maintained in vivo for long periods (Hadju et al. 1981; Sharkey et al. 1978; Llombart-Bosch et al. 1989). To our knowledge no ESTH has been grafted into nude mice until now, unlike other PNET of soft tissue or melanomas (Fogh et al. 1980; Hata et al. 1984; Sharkey et al. 1985).

The present tumour was transplanted into athymic nude mice and has been maintained in xenografts for nine generations over a two-year period. In this time the tumour has grown in nearly 100% of the animals, progressively appearing as subcutaneous, soft brown fleshy masses. Metastasis occurred in only one case, into the lung.

Progressive cell heterogeneity has been found in this transplanted neoplasm; the original tumour was apparently composed of only small round-to-spindle-shaped cells, devoid at histology of any pigmentation, while in the nude mice system small spindle cells appeared containing melanin as well as large epithelioid cells heavily loaded with melanin located both in the perikaryon and in the cell projections. Immunohistochemistry of the transplants revealed positivity for NSE and S-100 protein in paraffin-embedded material. All cells, both small spindle and large epithelioid, were NSE-positive, while S-100 protein appeared limited to the large epithelioid, being associated with the presence of melanin-pigment. As is known, melanocytic lesions are classically NSE- and S-100 protein positive, but negative for neurofilaments and cytokeratin (Dhillon et al. 1982; Gatter et al. 1984; Rode and Dhillon 1984; Gown et al. 1986).

The EM features of this tumour combine both neuroectodermal and melanocyte characteristics, resembling pigmented olfactory neuroblastoma (Curtis and Rubinstein 1982) and other PNET containing melanin pigment (Hahn et al. 1976; Dehner et al. 1979; Lowman and Livolsi 1980; Mullins 1980; Navas-Palacios 1980; Johnson et al. 1983; Krausz et al. 1984; Erlandson 1985; Chadarevian et al. 1987; Stirling et al. 1988). Two types of melanin pigment may be produced: neural crest type melanin with presence of premelanosomes in various stages of maturation, and neuromelanin, which is rich in lipids and connected with lysosomes, as seen in the central and sympathetic nervous systems (Mullins 1980) as well as in some carcinoid neoplasms (Cebelin 1980). This type of neuromelanin was found in the pigmented ESTH previously described by Curtis and Rubinstein (1982).

In this neoplasm we have detected neural crest type melanosomes in various stages of maturation, as well as dense-core membrane-bound granules of neurosecretory type. Both structures were found within the same tumour cells but rarely in the original neoplasm, and more abundantly in successive tumour transfers. The transplanted neoplasm showed a progressive increase in pigment content connected to a greater cell polymorphism, with the presence of large epithelioid cells loaded with melanin.

Cytogenetic analysis confirms the presence of both types of differentiation within the same neoplasm, showing both melanoma and ESTH markers. The chromosome 1 abnormality, in particular, displayed a del(1) (p11) similar to that observed in human malignant melanoma (Rey et al. 1985; Balaban et al. 1986; Pedersen et al. 1986). Other markers have been proposed for malignant melanoma and have been found in this case: a del(6) and extra copies of chromosome 7 were also present. However, the presence of a marker dup(14) (q24, q32) has been described by Whang-Peng et al. (1987) and Cavazzana et al. (1988) in ESTH. Such duplication has been seen in this case and therefore could be considered to be a necessary

chromosomal change for ESTH. The preferential involvement of the chromosome 1 (3 aberrations) in this tumour should also be pointed out, a fact which links this neoplasm not only with melanoma but also with neuroblastoma (Sandberg and Turc-Carel 1987).

The morphological and biological parameters herein discussed confirm not only the neural nature of this neoplasm with its PNET expression, but also the existence of a dual differentiation within the neoplastic cells (ESTH and melanoma). These findings support the existence of an unusual variant of neuroectodermal tumour, which should be designated "pigmented esthesioneuroblastoma".

References

Axe S, Kuhajda FP (1987) Esthesioneuroblastoma. Intermediate filaments, neuroendocrine and tissue-specific antigens. Am J Clin Pathol 88:139–145

Balaban GB, Herlyn M, Clark WH Jr, Nowell C (1986) Karyotypic evolution of human malignant melanoma. Cancer Genet Cytogenet 19:113–122

Berger L, Luc G, Richard D (1924) L'esthesioneuroepitheliome olfatif. Bull Assoc Franc Etude Cancer 13:410–421

Burns J (1982) The unlabelled antibody peroxidase-antiperoxidase method: In: Bullock GR, Petrusz P (eds) Techniques in Immunohistochemistry. Academic Press, London, pp 91–105

Cavazzana AO, Navarro S, Noguera R, Reynolds PC, Triche T (1988) Olfactory neuroblastoma is not a neuroblastoma but is related to primitive neuroectodermal tumor (PNET) In: Evans AE, D'Angio GJ, Knudson AG, Seeger RC (eds) Advances in neuroblastoma research 2. Prog Clin Biol Res, vol. 271. Alan R. Liss, Inc., New York, pp 463–474

Cebelin MS (1980) Melanocytic bronchial carcinoid tumors. Cancer 46:1843–1848

Chadarevian JP, Montes MJ, O'Gorman AM, Freeman AR (1987) Maturation of cerebellar neuroblastoma into ganglioneuroma with melanosis. A histologic, immunocytochemical and ultrastructural study. Cancer 59:69–76

Chaudhry AP, Haar JG, Koul A, Nickerson PA (1979) Olfatory neuroblastoma (esthesioneuroblastoma). A light and ultrastructural study of two cases. Cancer 44:564–579

Choi HSH, Anderson PS (1985) Immunohistochemical diagnosis of olfactory neuroblastoma. J Neuropathol Exp Neurol 44:18–31

Curtis JL, Rubinstein LJ (1982) Pigmented olfatory neuroblastoma. A new example of melanotic neuroepithelial neoplasm. Cancer 49:2136–2143

Dehner LP, Sibley RR, Sauk JJ, Vickers RA, Nesbit ME, Leonard AS, Waite DE, Neeley JE, Ophoven J (1979) Malignant melanotic neuroectodermal tumor of infancy: A clinical, pathological, ultrastructural and tissue-culture study. Cancer 43:1389–1420

Dhillon AP, Rode J, Leathem A (1982) Neuron specific enolase: an aid to the diagnosis of melanoma and neuroblastoma. Histopathology 6:81–92

Erlandson RA (1985) Melanotic Schwannoma of spinal nerve origin. Ultrastr Pathol 9:123–129

Fogh J, Tiso J, Sharkey E, Fogh JM, Daniels W (1980) Twenty-three new human tumor lines established in nude mice. Exp Cell Biol 48:229–239

Gatter KC, Pulford KAF, Vanstapel MJ, Roach B, Mortimer P, Woolston RE, Taylor J, Lane EB, Mason DY (1984) An immunohistochemical study of benign and malignant skin tumors: epithelial aspects. Histopathology 8:209–227

Gown AM, Vogel AM, Hoak D, Gough F, McNutt MA (1986) Monoclonal antibodies specific for melanocytic tumors distinguish subpopulation of melanocytes. Am J Pathol 123:195–203

Hadju SJ, Lemos LB, Kozakewich H, Helson L, Beattie E (1981) Growth pattern and differentiation in human soft-tissue sarcomas in nude mice. Cancer 47:90–98

Hahn JF, Netsky MG, Butter AB, Sperber DD (1976) Pigmented ganglioneuroblastoma: relation of melanin and lipofuscin to Schwannomas and other tumors of neural crest origin. J Neuropathol Exp Neurol 35:393–403

Hata J, Ueyama Y, Tamaoki N (1984) Transplantability and differentiating capacity of human tumors of neuroblastic, nephroblastic and germ cell origin in nude mice. In: Sordat B (ed) Immunedeficient animals. S Karger AG, Basel, pp 291–297

Homzie MJ, Elkon D (1980) Olfatory esthesioneuroblastoma. Variables predictive of tumor control and recurrence. Cancer 46:2509–2513

Hsu A-M, Raine L, Fange H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique; a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:557–580

ISCN (1985) An international system for human cytogenetic nomenclature. S Karger AG, Basel

Johnson RE, Scheithauer BW, Dahlin DC (1983) Melanotic neuroectodermal tumor of infancy. A review of seven cases. Cancer 52:661–666

Kadish S, Goodman M, Wang CC (1976) Olfactory neuroblastoma. A clinical analysis of 17 cases. Cancer 37:1571–1576

Kahn LB (1974) Esthesioneuroblastoma: a light- and electron-microscopic study. Hum Pathol 3:364–371

Krausz T, Azzopardi JE, Pearse E (1984) Malignant melanoma of the sympathetic chain: with a consideration of pigmented nerve sheath tumors. Histopathology 8:881–894

Llombart-Bosch A, Lacombe MJ, Contesso G, Peydro-Olaya A (1987) Small round blue cell sarcoma of bone mimicking atypical Ewing's sarcoma with neuroectodermal features. An analysis of five cases with immunohistochemical and electron microscopical support. Cancer 60:1570–1582

Llombart-Bosch A, Carda C, Boix J, Pellin A, Peydro-Olaya A (1989) Value of nude mice xenografts in the expression of cell heterogeneity of human sarcomas of bone and soft tissue. Pathol Res and Pract

Llombart-Bosch A, Lacombe MJ, Peydro-Olaya A, Perez-Bacete M, Contesso G (1988) Malignant peripheral neuroectodermal tumors of bone other than Askin's neoplasm: characterization of 14 new cases with immunohistochemistry and electron microscopy. Virchows Arch [A] 412:421–430

Lowman RM, Livolsi VA (1980) Pigmented (melanotic) Schwannomas of the spinal canal. Cancer 46:391–397

Mackay B, Luna MA, Butler TJ (1976) Adult neuroblastoma. Electron microscopical observation of nine cases. Cancer 37:1334–1351

Micheau C, Guerinot F, Bohvon C, Brugere J (1975) Dopamine beta-hydroxilase and cathecholamine in an olfatory esthesioneuroblastoma. Cancer 35:1309–1312

Miller DC, Goodman M, Pilch BZ, Shi SR, Dickersin GR, Halpern H, Norris CM (1984) Mixed olfactory neuroblastoma and carcinoma. A report of two cases. Cancer 54:2019–2018

Mills SE, Frierson HF (1985) Olfactory neuroblastoma. A clinicopathological study of 21 cases. Am J Surg Pathol 9:317–327

Mullins JD (1980) A pigmented differentiating neuroblastoma: A light- and ultrasturctural study. Cancer 46:522–528

Navas-Palacios JJ (1980) Malignant melanocytic neuroectodermal tumor. Cancer 46:529–536

Newbill ET, Johns ME, Cantrell RW (1985) Esthesioneuroblastoma: Diagnosis and management. South Med J 78:275–282

Ng HK, Poon WS, Poon WS, South JR (1988) Intracranial olfactory neuroblastoma mimicking carcinoma: report of two cases. Histopathology 12:393–403

Osamura RY, Fine G (1976) Ultrastructure of esthesioneuroblastoma. Cancer 38:173–179

Osborn M, Altmannsberger M, Shaw G, Schauer A, Weber K (1982) Various sympathetic derived human tumors differ in neurofilament expression. Virchows Arch [B] 40:141–146

Pedersen MI, Bennett JW, Wang N (1986) Non-random chromosome structural aberrations and oncogene loci in human malignant melanoma. Cancer Genet Cytogenet 20:11–27

Rey JA, Bello MJ, Campos JM, Ramos MC, Benitez J (1985) Cytogenetic findings in a human malignant melanoma metastatic to the brain. Cancer Gen Cytogen 16:179–183

Rode J, Dhillon AP (1984) Neuron specific anolase and S-100 protein as possible prognostic indicators in melanoma. Histopathology 8:1041–1052

Sandberg AA, Turc-Carrel CL (1987) The cytogenetics of solid tumors. Cancer 59:387–395

Sharkey FE, Fogh JM, Hajdu SJ, Fitzgerald P, Fogh JM (1978) Experiences in surgical pathology with human tumor growth in nude mice. In: Fogh J, Giovanella S (eds) The nude mouse in experimental and clinical research, vol. I, Academic Press, New York pp 187–214

Sharkey FE, Spicer JM, Fogh J (1985) Changes in histological differentiation of human tumors transplanted into athymic nude mice: A morphometric study. Exp Cell Biol 53:100–106

Silva EG, Butler JJ, Mackay B, Goepfert H (1982) Neuroblastomas and neuroendocrine carcinomas of the nasal cavity. A proposed new classification. Cancer 50:2388–2405

Sternberger LA, Hardy PH Jr, Cuculis JJ, Meyer HG (1970) The unlabelled antibody enzyme method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–333

Stirling RW, Powell G, Fletcher CDM (1988) Pigmented neuroectodermal tumor of infancy: an immunohistochemical study. Histopathology 12:425–435

Taxy JB, Hidvegi DF (1977) Olfactory neuroblastoma. An ultrastructural study. Cancer 39:131–138

Trojanowski JQ, Lee V, Pillsbury N, Lee S (1982) Neuronal origin of human esthesioneuroblastoma demonstrated with anti-neurofilament monoclonal antibodies. N Engl J Med 307:159–161

Trojanowski JQ, Lee V, Leev MY, Schlaeper W (1984) An immunohistochemical study of human central and peripheral nervous system tumors, using monoclonal antibodies against neurofilaments and glial filaments. Hum Pathol 15:248–257

Venable JH, Goggenshau R (1965) A simplified lead citrate stain for use in electron microscopy. J Cell Biol 25:407–408

Vollruth N, Altmannsberger M, Hunneman DM et al. (1984) Esthesioneuroblastoma: Ultrastructural, immunohistochemical and biochemical investigation of one case. Arch Otorhinolaryngol 239:133–144

Whang-Peng J, Freier RE, Knutsen T, Nanfro JJ, Gazdar A (1987) Translocation t(11;22) in esthesioneuroblastoma. Cancer Genet Cytogenet 29:155–157

Received July 5, 1988 / Accepted October 3, 1988