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REVIEW

Morphological and immunophenotypic variations in malignant melanoma

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Morphological and immunophenotypic variations in malignant melanoma

A variety of cytomorphological features, architectural patterns and stromal changes may be observed in malignant melanomas. Hence, melanomas may mimic carcinomas, sarcomas, benign stromal tumours, lymphomas, plasmacytomas and germ cell tumours. Melanomas may be composed of large pleomorphic cells, small cells, spindle cells and may contain clear, signet-ring, pseudolipoblastic, rhabdoid, plasmacytoid or balloon cells. Various inclusions and phagocytosed material may be present in their cytoplasm. Nuclei may show bi- or multi-nucleation, lobation, inclusions, grooving and angulation. Architectural variations include fasciculation, whorling, nesting, trabeculation, pseudoglandular/pseudopapillary/pseudofollicular, pseudorosetting and angiocentric patterns. Myxoid or desmoplastic changes and very rarely pseudoangiosarcomatous change, granulomatous inflammation or osteoclastic giant cell response may be seen in the stroma. The stromal blood vessels may exhibit a haemangiopericytomatous pattern, proliferation of glomeruloid blood vessels and perivascular hyalinization. Occasionally, differentiation to nonmelanocytic structures (Schwannian, fibro-/myofibroblastic, osteocartilaginous, smooth muscle, rhabdomyoblastic, ganglionic and ganglioneuroblastic) may be observed. Typically melanomas are S100 protein, NKIC3, HMB-45, Melan-A and tyrosinase positive but some melanomas may exhibit an aberrant immunophenotype and may express cytokeratins, desmin, smooth muscle actin, KP1 (CD68), CEA, EMA and VS38. Very rarely, neurofilament protein and GFAP positivity may be seen.

Keywords: immunophenotypic aberration, malignant melanoma, morphological variation

Introduction

Most malignant melanomas, especially of the skin, are easily diagnosed but some, particularly those presenting as noncutaneous primaries or as metastatic disease may closely mimic other tumours (Table 1). As the late Arnold Levene[1] remarked in a review article published 20 years ago, 'Among the difficult diagnostic fields in histopathology melanocytic tumours have achieved a notoriety'.

Based on personal experience of over 1200 cases of melanoma seen in this department over the last 10 years, together with a review of the literature, we discuss the bewildering variations in morphology and immunophenotype which can, on occasion, mislead the most experienced pathologist as well as the tyro, and we illustrate the least well-known variants.

The variations in cytomorphology, architecture and stromal components which may be observed in melanocytic tumours are listed in Table 2. Sometimes a combination of these morphological changes are seen in the same tumour.

Variation in size of cells

It is well known that some melanomas are composed of large pleomorphic cells and may mimic large-cell carcinomas, anaplastic large-cell lymphomas or

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Table 1. Malignant melanomas may mimic the following non-melanocytic tumours

Carcinomas
Squamous cell carcinoma – poorly differentiated, spindle cell and pseudo-angiosarcomatous variants
Adenocarcinoma – poorly differentiated and papillary variants
Signet ring cell carcinoma
Extramammary Paget's disease
Sebaceous carcinoma
Large-cell undifferentiated carcinoma
Clear-cell carcinoma
Small-cell carcinoma
Metaplastic carcinoma with bone and cartilage formation
Neuroendocrine tumours – including Merkel cell carcinoma, paraganglioma and olfactory neuroblastoma
Sarcomas
Fibrosarcoma
Leiomyosarcoma
Malignant fibrous histiocytoma, atypical fibroxanthoma, myxofibrosarcoma
Dermatofibrosarcoma protuberans
Malignant peripheral nerve sheath tumour
Synovial sarcoma
Rhabdomyosarcoma
Epithelioid sarcoma
Epithelioid angiosarcoma
Kaposi's sarcoma
Haemangiopericytoma
Liposarcoma (myxoid and pleomorphic variants)
Alveolar soft part sarcoma
Extraskeletal Ewing's sarcoma/peripheral neuroectodermal tumour
Osteo and chondrosarcoma
Gastrointestinal autonomic neuronal tumour (plexosarcoma)
Malignant lymphomas (particularly the anaplastic large-cell Ki-l lymphoma) and rarely plasmacytoma and dendritic reticulum cell sarcoma
Germ cell tumour – particularly dysgerminoma and metastatic or extra-testicular seminoma
Benign tumours
Benign fibrous histiocytoma
Reticulohistiocytoma
Xanthoma
Cellular neurothekeoma
Neurofibroma
Schwannoma
Leiomyoma
Fibromatosis

pleomorphic sarcomas. However, the existence of a small-cell melanoma[2–14] is not widely known. These tumours are composed of rather monomorphic small cells (size similar to the cells of an intradermal naevus or lymphoid cells) with markedly hyperchromatic round or oval nuclei, usually small inconspicuous nucleoli and scanty cytoplasm (Figure 1). Nucleoli may be prominent in some tumours. Angular nuclei and nuclear moulding may be seen. Mitoses and fragments of nuclear debris are commonly observed. The cells are usually arranged in sheets but vague nesting may be present. Melanin is usually scanty or absent. Perivascular concentration of

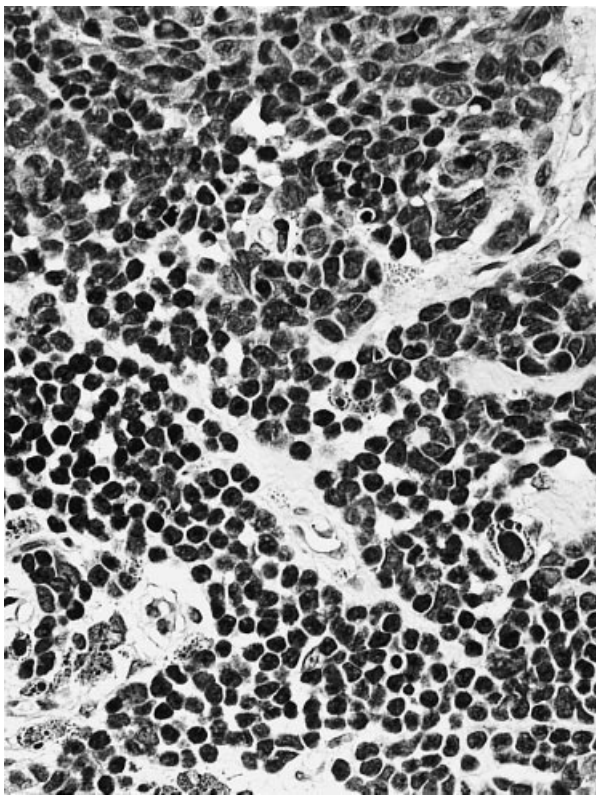


Figure 1. A small-cell melanoma of skin. The cells contain hyperchromatic nuclei and scanty cytoplasm (H & E).

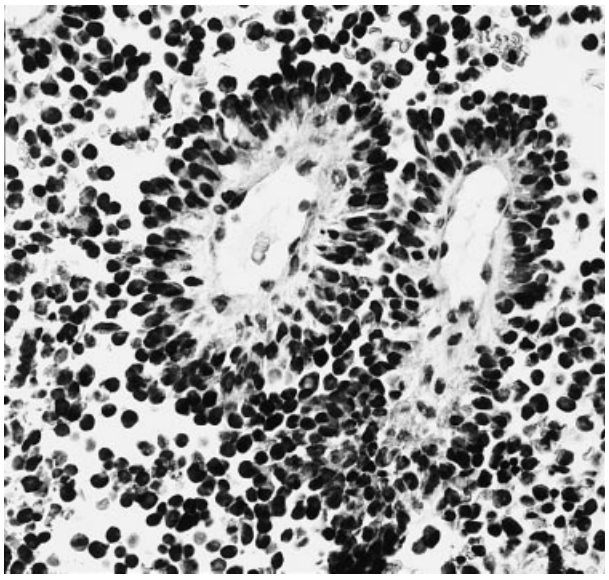


Figure 2. Metastatic small-cell melanoma in the bone marrow showing perivascular pseudo-rosettes (H & E).

the cells may give rise to pseudorosette-like structures in metastatic lesions (personal observation) (Figure 2). Monomorphism of the cells and scantiness of the cytoplasm are the two characteristic features of these tumours which separate them from conventional melanomas. This rare variant has been described both in children and adults and there is no sex predilection. In children they usually arise in the dermal component of large or giant congenital naevi.[2,3,9] Some authors[2] described these neoplastic cells as lymphoblast-like whilst Reed[9] used the term 'melanoblastomas' for such tumours.

Recently Barnhill *et al.*[11,13] described five cases of cutaneous small-cell melanoma in children, only one of which developed in a congenital naevus and the rest were *de novo* lesions. They were localized exclusively to the scalp, were thick tumours (mean Breslow thickness 6.7 mm) and were associated with aggressive behaviour; all patients died of tumour. Two of these tumours exhibited papillomatous or verrucous epidermal surfaces and mimicked papillomatous naevi. In adults, primary *de novo* cutaneous melanomas of all types (nodular, superficial spreading, lentigo maligna and acral lentiginous melanomas) and mucosal melanomas at various sites may be composed almost entirely of small cells but in some cases islands of small cells are present in a typical epithelioid or spindle cell melanoma (personal observation). Interestingly, review of the existing literature suggests that small-cell melanomas are more commonly seen within the nasal cavity and paranasal sinuses.[4,7,14]

Sometimes metastatic melanomas are composed entirely of small cells; Fitzgibbons *et al*. and Young and Scully described such tumours in the ovary.[5,6] Attanoos and Griffiths[8] documented a case of metastatic small-cell melanoma in the stomach which mimicked a primary gastric lymphoma. Intraglandular melanoma cells in this case produced a picture reminiscent of lymphoepithelial lesions.

In the skin these tumours may be mistaken either for benign naevocytic lesions when they are small, symmetrical and nested or for malignant small round cell tumours (such as Merkel cell carcinoma, metastatic small-cell carcinoma, lymphomatous/leukaemic deposits, PNET, etc.) when they are diffuse, infiltrative and ulcerated with an inconspicuous junctional component. In cases of mucosal or metastatic small-cell melanomas, other small-cell tumours such as rhabdomyosarcomas, small-cell squamous carcinomas and olfactory neuroblastomas also enter the differential diagnosis.

To differentiate a naevoid small-cell melanoma from a cellular compound naevus it is helpful to look for atypical junctional activity, pagetoid spread, poor nesting of the dermal component, subtle nuclear atypia with

Table 2. Morphological variations in melanomas. Some melanomas show a combination of these aberrant morphological features

Variation in size of cells
Large cells
Small cells
Variation in shape of cells
Polygonal
Spindle
Dendritic
Variation in cytoplasmic features
Clear cells
Signet-ring cells
Pseudolipoblastic cells
Rhabdoid cells
Balloon cells
Inclusions and phagocytosed material:
Hyaline
Erythrocytic
Neutrophilic
Tumour cells
Variation in nuclear features
Bi- and multi-nucleation
Lobation
Plasmacytoid features
Intranuclear cytoplasmic inclusions
Nuclear grooving and angulation
Variations in architecture
Fasciculation
Whorling
Nesting
Trabeculation
Pseudoglandular/pseudopapillary/pseudofollicular pattern
Pseudorosetting and angiocentric pattern
Myxoid change in the stroma
Stromal desmoplasia and neurotropism
Changes in stromal vascularity
Prominent vascular proliferation in the stroma
Herniation of tumour cells in vessels as seen in MPNST
Haemangiopericytomatous pattern
Proliferation of glomeruloid vascular structures (as in neuroendocrine or glial tumours)
Perivascular hyalinization
Angiomatoid/pseudoangiosarcomatous pattern
Associated granulomatous inflammation
Osteoclastic giant cells in the stroma

Table 2. Continued

Differentiation towards nonmelanocytic elements
Schwannian
Fibroblastic/myofibroblastic
Smooth muscle
Rhabdomyoblastic
Osteocartilaginous
Ganglionic/ganglioneuroblastic

marked hyperchromatism and a coarse chromatin pattern, mitotic activity in the deeper part of the lesion, apoptosis and lymphocytic infiltration. HMB-45 positivity in the deeper part of the lesion is another worrying sign. Kossard and Wilkinson[12] have suggested that counting of AgNORs (nucleolar organizer regions) may help to differentiate a small-cell naevoid melanoma from an ordinary naevus. The average number of AgNORs per nucleus in 10 small-cell melanomas studied in their series was 5.83 (SD ± 1.69) compared to 2.71 (SD ± 0.50) for the 10 dermal naevi examined. To differentiate a small-cell melanoma from other malignant small blue cell tumours one should rely on the demonstration of melanin pigment, positivity for specific melanocytic markers and negative staining for cytokeratin, lymphoid markers, desmin and neuronal/neuroendocrine markers.

If necessary, electron microscopy should be performed to demonstrate melanosomes.

Variation in shape of cells

Spindling of cells in malignant melanomas is a common and well known occurrence which may lead to the misdiagnosis of a melanoma as a sarcoma[1,15] or sarcomatoid carcinoma. In our experience they are commonly misdiagnosed as MFH, MPNST or leiomyosarcoma. When dealing with a malignant spindle cell tumour at any site, one should always keep the possibility of a melanoma in mind and should include melanocyte markers in the immunopanel. Electron microscopy may also be useful in such a situation. However, differentiation of a spindle cell or desmoplastic melanoma from a MPNST may in some cases be extremely difficult if not impossible, as these two tumours share many morphological features such as fasciculation, whorling, nuclear palisading, dendritic cell morphology, wavy nuclei, geographical areas of necrosis with palisading of cells around the necrotic foci, herniation of tumour cells into vascular lumina, and perivascular hyalinization. Melanoma and MPNST are likely to be S100 protein positive but many spindle cell/desmoplastic melanomas are HMB-45 and Melan-A negative. Ultrastructurally, melanosomes are hard to find in spindle cell desmoplastic melanomas and some of these tumours may, in fact, exhibit true Schwannian differentiation[16] with cell processes and formation of well developed basal lamina. The presence of an in-situ melanoma in the overlying epidermis in a cutaneous lesion, a previous history of excision of a melanoma, diffuse S100 protein positivity, the presence of melanin pigment, prominent nucleoli and the presence of large epithelioid or polygonal cells mingling with spindle cells would favour a melanoma.

Melanomas with large polygonal cells may mimic large-cell carcinomas, anaplastic large-cell lymphomas and some sarcomas such as epithelioid sarcoma, epithelioid angiosarcoma, pleomorphic rhabdomyosarcoma and the epithelioid variant of MPNST.

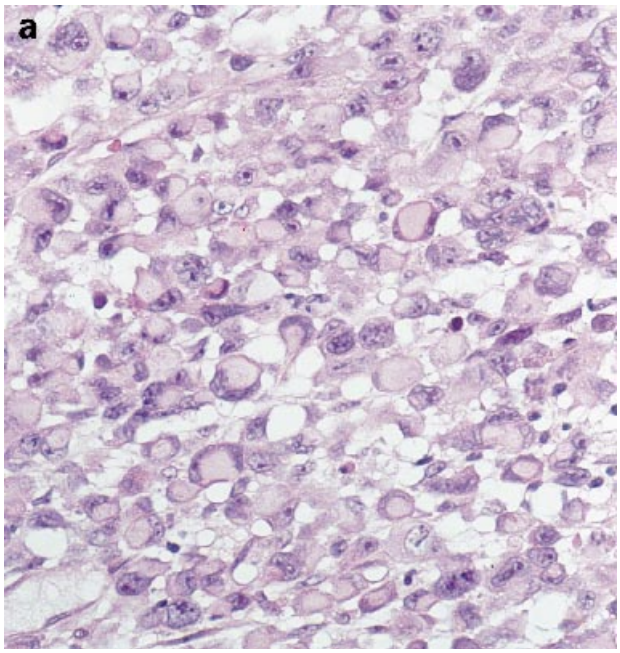
Dendritic morphology of malignant cells is usually seen in the in-situ component of acral lentiginous and mucosal malignant melanomas but is uncommon in invasive tumours.

Variation in cytoplasmic features

Both primary cutaneous/mucosal and metastatic melanomas may contain glycogen-rich clear cells[17–19] and may mimic clear-cell carcinomas or germ cell tumours. We have seen a case of glycogen-rich clear-cell metastatic amelanotic melanoma in the ovary of a young woman which was initially misdiagnosed as a dysgerminoma. Rare examples of the clear-cell type of spindle cell melanoma in the skin may mimic clear-cell leiomyosarcoma or clear-cell dermatofibroma. Melanomas of soft parts (so-called clear-cell sarcomas) are well known for their glycogen-rich clear-cell component.[20]

Signet-ring cell melanoma[4,21–26] is another rare morphological variant which closely resembles a signet-ring cell carcinoma, a signet-ring lymphoma or a liposarcoma. The signet-ring morphology is usually produced by accumulation of vimentin filaments in the

cytoplasm which tends to displace the nucleus to the periphery and indent it to a semilunar shape (Figure 3a,b). Rarely, signet ring change occurs due to the formation of an intracytoplasmic vacuole (personal observation) (Figure 4a,b). The nucleolus remains prominent in these cells. Signet-ring change is usually seen in recurrent or metastatic lesions and is usually focal but may be diffuse. These cells do not contain



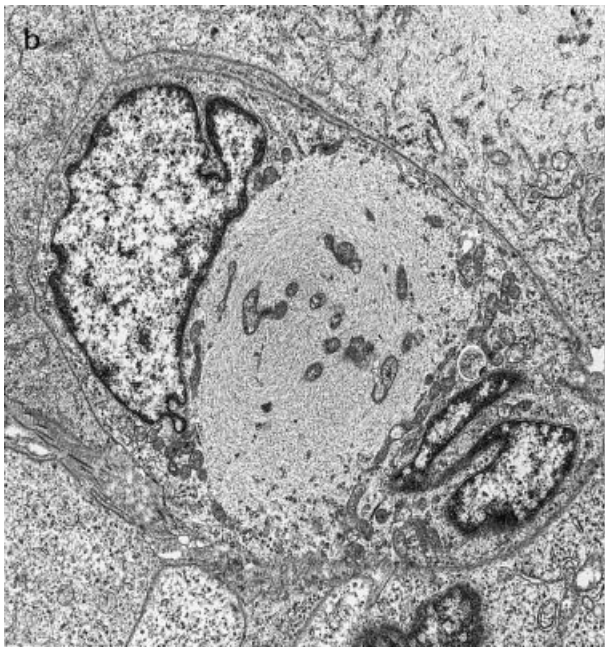


Figure 3. a, Signet-ring cell melanoma. Eosinophilic cytoplasmic globules composed of vimentin filaments displace nuclei to the periphery (H & E). **a,** Tumour cell of signet-ring morphology with crescentic nucleus indented by a mass of intermediate filaments containing entrapped mitochondria. Electron micrographs × 7000 (Micrograph courtesy of Brian Eyden, Christie Hospital, Manchester, UK).

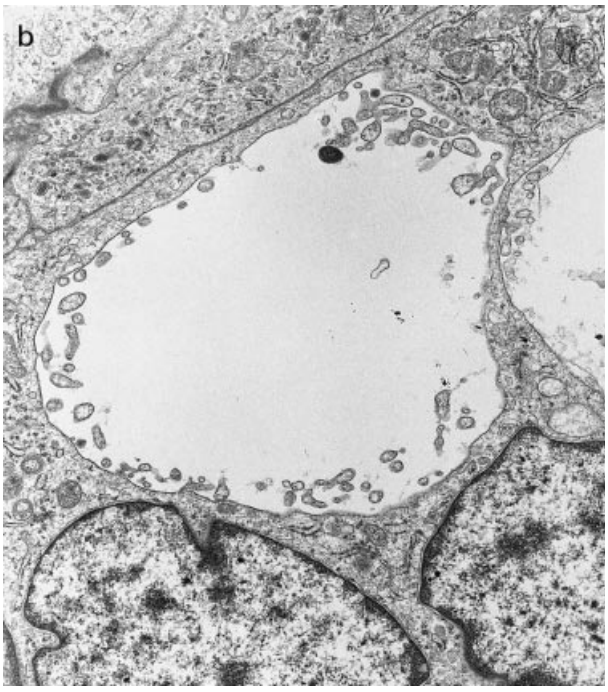


Figure 4. a, Signet-ring cell melanoma containing empty cytoplasmic vacuoles. (H & E). **b,** Electron micrograph showing a membrane-limited vacuole displacing nuclei to one side of the cell. The vacuole bears irregular processes, which are not true glandular microvilli (× 9000).

mucin, fat or glycogen but may contain granular diastase-resistant PAS-positive material. In difficult cases the true nature of the tumour cells can be established by appropriate immuno-stains although one should be aware of the fact that a few cases of S100 protein-negative signet-ring cell melanomas have been recorded in the literature.[22,23]

Occasionally the presence of multiple empty vacuoles within the cytoplasm with scalloping of nucleus may impart a pseudolipoblastic appearance[15] and, in a metastatic melanoma in the soft tissue with pleomorphic cells or myxoid stroma, this may lead to the erroneous diagnosis of a pleomorphic or myxoid liposarcoma. Rarely, a pseudolipoblastic appearance may be produced in a myxoid melanoma due to the presence of alcian blue positive mucinous material within the cytoplasm of the neoplastic melanocytes (personal observation).

Rhabdoid change[27–29] due to the presence of large glassy hyaline inclusions in the cytoplasm with eccentric round or irregular nuclei and prominent nucleoli is a rare phenomenon. The inclusions usually represent paranuclear whorls of intermediate filaments and this variant is therefore pathogenetically related to the more common form of signet-ring melanomas. In some cases the rhabdoid appearance is caused by a collection of mitochondria and dilated rough endoplasmic reticulum that contains microtubular arrays.[28] A rhabdoid melanoma may be mistaken for a rhabdomyosarcoma or an extrarenal rhabdoid tumour. The immunohistochemical features of these tumours may be misleading. Bittesini *et al.*[27] documented a case of rhabdoid metastatic melanoma in a lymph node in which the cells exhibited vimentin, cytokeratin and desmin positivity but there were no ultrastructural features of rhabdomyoblastic differentiation. Borek *et al.*[29] have described three cases of primary rhabdoid melanoma of skin, one of which showed cytokeratin and focal α-SMA positivity. A diminution in the expression of S100 protein and HMB-45 has been observed in some cases of rhabdoid melanoma which may also lead to diagnostic difficulty.[28]

In balloon cell melanomas[30–35] the neoplastic cells appear large, polygonal or round and contain abundant finely granular, reticulated or vacuolated cytoplasm with delicate, focally disrupted cytoplasmic membranes. The nuclei may be central or eccentric and show a mild to moderate degree of hyperchromatism and pleomorphism with one or two eosinophilic nucleoli. Cells with larger vacuoles and scalloped nuclei mimicking lipoblasts may be seen (Figure 5). The cytoplasm is devoid of glycogen and may show fine melanization. Rare mitoses are seen. Usually balloon cell change occurs focally in a conventional melanoma but rarely the entire tumour may be composed of these cells and the tumour may simulate a clear-cell carcinoma, sebaceous carcinoma or a lesion of foamy histiocytes such as a xanthoma or Rosai–Dorfman disease. Balloon cell melanomas are S100 protein and HMB-45 positive and negative for cytokeratin. Ultrastructurally the cells show numerous intracytoplasmic vacuoles which probably represent enlarged and coalescent melanosomes.

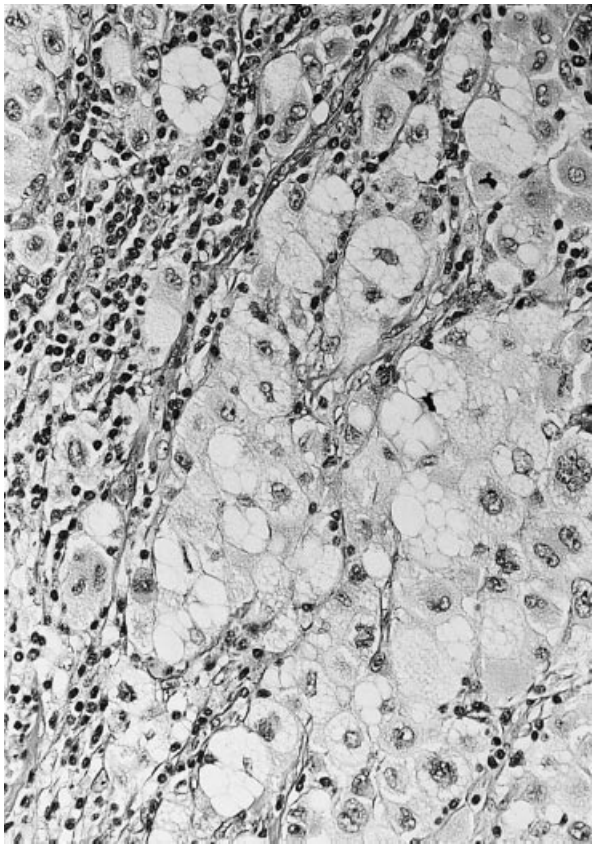


Figure 5. Metastatic balloon cell melanoma in a lymph node containing pseudolipoblastic cells (H & E).

Melanoma cells may contain a variety of intracytoplasmic inclusions/phagocytosed material of which hyaline eosinophilic PAS+ globules are the most common; similar globules are present in a variety of epithelial and mesenchymal tumours.[36,37] The precise nature of these globules is uncertain; they may either represent engulfed cellular material from necrotic or apoptotic cells or degenerated red cells.[37] Phagocytosed erythrocytes and neutrophils are rarely seen in melanoma cells.[38,39] One tumour cell phagocytosing another is an extremely uncommon phenomenon.[39]

Variations in nuclear features

Bi- and multi-nucleation can be seen in pleomorphic

melanomas and Reed–Sternberg-like cells may occasionally be present in these tumours. Melanomas with multiple and lobated nuclei may closely resemble anaplastic large-cell lymphomas, large-cell carcinomas (particularly of renal, adrenocortical or pulmonary origin) or pleomorphic sarcomas. We have encountered a few metastatic melanomas, one case of primary intranasal melanoma and one cutaneous melanoma, resembling plasmacytoma. These tumours contained aggregates of plasmacytoid cells with eccentric nuclei showing coarse chromatin, inconspicuous nucleoli and abundant cytoplasm with pale paranuclear zones (Figure 6). The tumour cells in all cases were positive for melanocytic markers and negative for light chains. In this context, one should bear in mind that the so-called plasma cell marker VS38 is not useful since this antibody stains a large proportion of malignant melanomas.[40]

Intranuclear cytoplasmic pseudoinclusions are commonly seen in melanomas and, although non-specific, are a useful diagnostic clue. Nuclear grooving and angulation have been described in metastatic melanomas in the ovary[5,6] and these tumours superficially resembled adult granulosa cell tumours. We have seen a recurrent cutaneous melanoma composed of centrocyte-like cells with cleaved or grooved nuclei and inconspicuous nucleoli (Figure 7).

Variations in architecture

Fasciculation and whorling[1,15] are seen in spindle cell sarcomatoid melanomas. Nesting is also a common phenomenon but a trabecular pattern[1] is only rarely seen. An amelanotic melanoma with a nested and trabecular pattern containing a relatively uniform population of epithelioid cells may be mistaken for a neuroendocrine tumour. Melanomas are commonly Grimelius positive which may further compound the problem, but stains for chromogranin and synaptophysin, are usually negative in melanocytic tumours. A nested melanoma with large cells may also mimic an alveolar soft part sarcoma. Rarely, primary and metastatic melanomas contain pseudoglandular or pseudopapillary structures mimicking adenocarcinoma.[1,4]

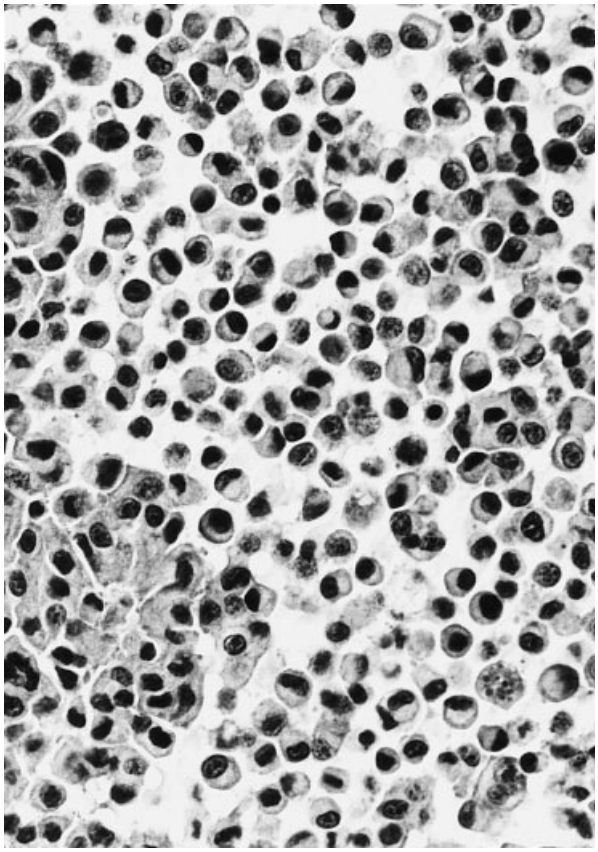


Figure 6. Metastatic melanoma containing plasmacytoid cells (H & E).

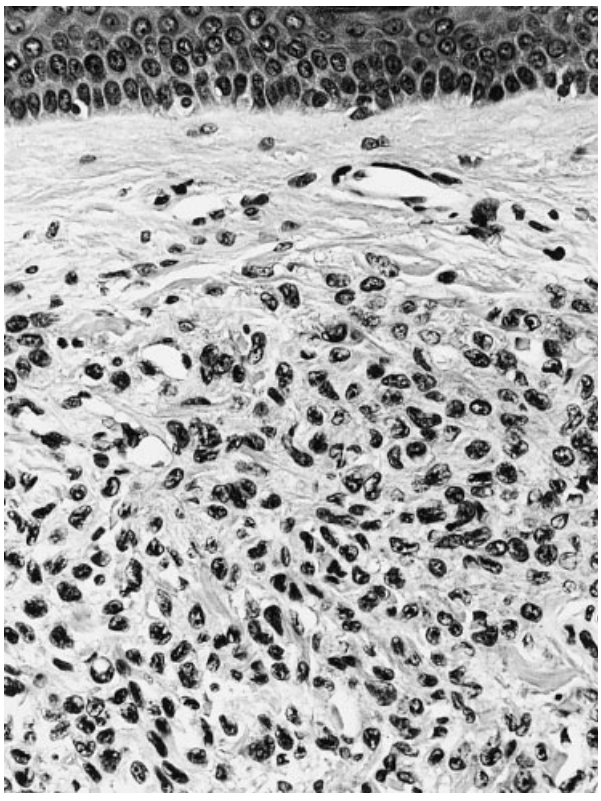


Figure 7. Recurrent cutaneous melanoma containing centrocyte-like cells (H & E).

Follicle-like structures have been described in ovarian metastases of malignant melanomas.[6] These structures were either empty or contained thin pink fluid or peripherally scalloped colloid-like material. Occasionally, melanomas contain pseudorosettes with malignant cells radially arranged around blood vessels. Rarely, cells of conventional melanomas and desmoplastic melanomas concentrate around and invade walls of large blood vessels producing a striking angiocentric and angioinvasive picture.

Myxoid change

In 1986 Bhuta *et al.*[41] described four cases of metastatic malignant melanoma which exhibited prominent myxoid change in the stroma. Subsequently several additional cases of primary cutaneous, mucosal and metastatic myxoid melanomas have been documented in the literature.[4,42–48] The myxoid change in the tumour may be focal or diffuse and in a primary cutaneous lesion this change may occur in any variant of melanoma. Myxoid change is also seen in desmoplastic melanomas. Myxoid melanomas may exhibit vague lobularity with rounded, pushing margins, individual lobules being surrounded by delicate fibrovascular septa. Paraseptal and perivascular concentration of cells may occur in some cases. The neoplastic cells in these tumours usually appear small, stellate or spindled and are arranged singly or in cords. Rarely pseudoglandular structures are seen. Some tumours contain large epithelioid cells. Scattered pseudolipoblastic cells may be noted. Small pools of mucin may be present producing a close resemblance to myxoid liposarcoma. Melanin pigment is usually scanty or absent. The stroma may be moderately vascular but a chicken wire type of vascularity is never seen. Most cases are S100 protein, NKIC3 and HMB-45 positive.

Myxoid melanomas should be distinguished from other myxoid tumours, such as mucinous adenocarcinomas, myxoid MFH/AFX, MFS, myxoid liposarcomas, nerve sheath myxomas, myxoid MPNST and extraskeletal myxoid chondrosarcomas.

Stromal desmoplasia and neurotropism

Minor degrees of stromal desmoplasia may be observed in some cases of conventional primary cutaneous, mucosal or metastatic melanomas but this phenomenon is prominent and diffuse in so-called desmoplastic malignant melanomas (DMM).[16,49–64] In these tumours the neoplastic cells themselves may undergo fibroblastic or myofibroblastic differentiation or may stimulate proliferation of stromal fibroblasts/myofibroblasts which is associated with marked collagenization of the intercellular matrix.

Although the pathological features of DMM have been well described in several excellent papers and monographs on melanocytic lesions, these tumours are still frequently misdiagnosed because of their protean and, at times, deceptively innocuous histological appearances. Clinically, they usually present as pigmented or nonpigmented indurated areas, plaques or nodules in the dermis or subcutis of elderly white individuals. Head and neck are common sites, although they can occur at other anatomical sites including mucosae. Rarely, a conventional melanoma may recur as a desmoplastic melanoma. There is no sex predilection and the size may vary from 4 to 60 mm in maximum diameter. Histologically, the tumours are composed of fascicles, sheets, nodules and strands of mildly to markedly atypical spindle cells which infiltrate the dermis and often extend to subcutis or deeper tissues with margins which are often ill-defined; satellite foci may be present.

The nuclei of the neoplastic cells may be hyperchromatic or vesicular with inconspicuous or prominent nucleoli and variable amounts of cytoplasm. The cells may resemble fibroblasts, Schwann cells or smooth muscle cells. The concentration of cells may vary markedly and some tumours may be quite paucicellular. Multinucleated tumour cells and scattered epithelioid malignant cells may be present. Mitotic activity is extremely variable but usually low. Atypical mitoses have been noted in mitotically active tumours. Most DMM are entirely amelanotic. Fibroblastic proliferation and collagenization of the stroma is prominent, although the degree may vary from area to area. In some cases there is also myxoid change. Elastosis of dermal collagen is commonly observed. Curvilinear arrangements of fusiform cells, storiform patterns, cellular whorls, osteogenesis and formation of small pseudoglandular or pseudovascular structures are additional but inconstant features. Neuroidal structures resembling small peripheral nerves or Meissner corpuscles may be seen. Small foci of necrosis may be present and infiltration by lymphocytes and formation of lymphoid nodules is a common feature. The overlying epidermis is usually intact and may show an in-situ melanoma, commonly of lentigo maligna type. Occasionally DMM arise from an acral lentiginous, mucosal lentiginous or a superficial spreading melanoma. In some cases no epidermal component is seen which is probably due to complete regression of the in-situ component or an origin from appendageal melanocytes. Many desmoplastic melanomas also exhibit neurotropism with perineural and endoneural invasion of nerve

fascicles by malignant cells.[52,56,58,62] These tumours may grow along the involved nerves for a considerable distance and eradication of the tumour becomes difficult. In the head and neck region DMM may extend into the brain along cranial nerves. Neurotropism may also be seen in rare cases of nondesmoplastic epithelioid cell melanomas.

DMM with an inconspicuous or absent in-situ component, mild atypia, low mitotic activity and fibroblastic, smooth muscle or Schwannian type features may mimic scar tissue, benign desmoplastic and/or neuronized naevi and benign dermal connective tissue tumours (such as benign fibrous histiocytoma, neurofibroma, Schwannoma, leiomyoma or fibromatosis). On the other hand, a cellular, mitotically active and cytologically atypical DMM may be mislabelled as a MPNST, AFX, DFSP, MFS, leiomyosarcoma, spindle cell angiosarcoma, Kaposi's sarcoma or spindle cell carcinoma. Careful scrutiny of the histological features and immunohistochemical stains should help to make the correct diagnosis. The vast majority of DMM are S100 protein positive (95–100% according to various series) which may be diffuse or patchy.[61–63] Some are NKIC3 positive but the majority are HMB-45 and Melan-A negative. They are negative for cytokeratin, CD34 and CD31. Smooth muscle actin (α-SMA) shows consistent positivity indicating presence of large numbers of myofibroblastic cells.[62,63] Ultrastructurally, premelanosomes are rarely found and the tumour cells may exhibit Schwannian, perineural or transitional features.[16,62] Fibroblastic and myofibroblastic cells are also present.[53,55]

Changes in stromal vascularity

Mucosal and metastatic melanomas usually tend to show prominent stromal vascularity with formation of both well formed and poorly formed blood vessels. Foci of stromal haemorrhage in these tumours are not uncommon. Occasionally, the neoplastic melanocytes invade walls of blood vessels and herniate into the lumen – a feature which has been described in MPNST. Prominent haemangiopericytomatous vascular proliferation[4,15] has been seen in rare cases and we have noted this phenomenon (Figure 8) in a uterine and mesenteric metastasis of a malignant melanoma from an unknown primary site. The tumour was initially misdiagnosed as a haemangiopericytomatous leiomyosarcoma but subsequent investigations showed lack of staining for α-SMA and muscle specific actin, occasional desmin positive cells, diffuse S100 protein and patchy HMB-45 positivity. Electron microscopy revealed premelanosomes within the tumour cells. Proliferation of glomeruloid blood vessels is another interesting but

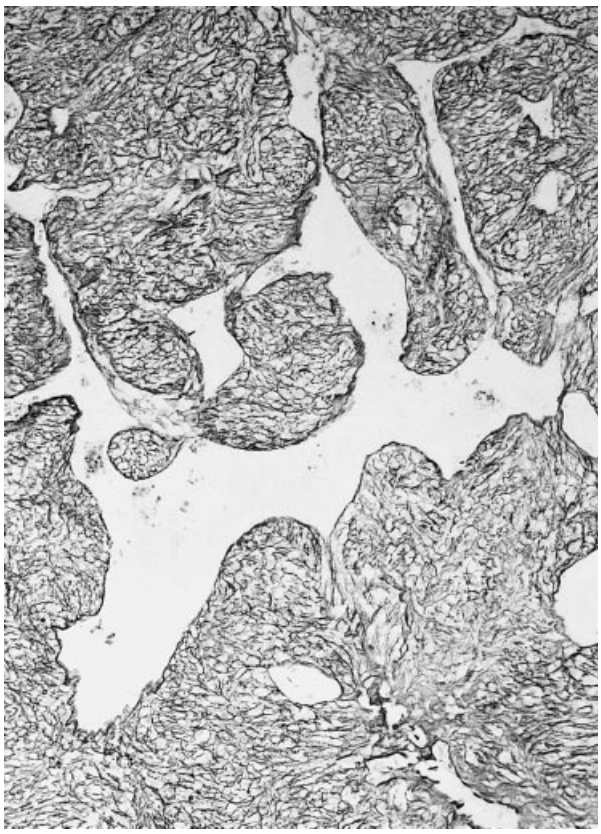


Figure 8. Reticulin stain showing haemangiopericytomatous vascular pattern in a metastatic melanoma.

extremely unusual vascular change which may be observed in melanomas. Gaudin and Rosai[65] provided a detailed description of this florid vascular lesion in neuronal and neuroendocrine tumours. Similar changes are also seen in high-grade astrocytomas. In a letter subsequently published in response to Gaudin and Rosai's article, it was pointed out that such vascular proliferations are not entirely specific for neuronal/neuroendocrine tumours and a case of metastatic melanoma in the pleura containing glomeruloid vessels was illustrated.[66] We have also encountered glomeruloid stromal vascular proliferation (Figure 9a,b) in two cases of melanoma, one of which was a primary palatal mucosal melanoma and the other was a metastatic lesion in the subcutis from a primary cutaneous melanoma. The changes were focal in both cases. It is possible that some melanomas produce potent angiogenic factors which may lead to vascular proliferation of different morphological types. Perivascular hyalinization has been described in cases of metastatic spindle cell melanoma in the lung mimicking Schwannian tumours.[67]

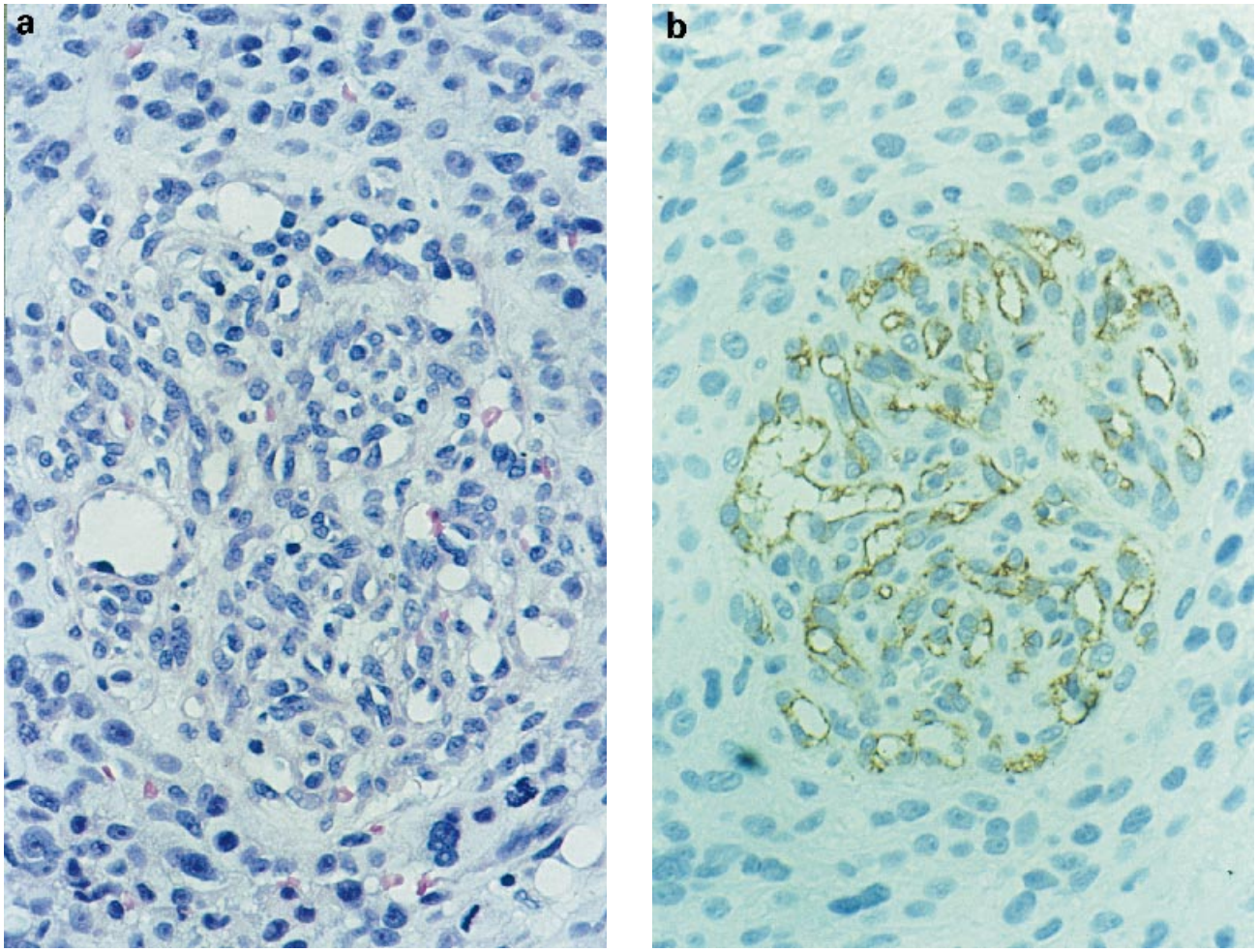


Figure 9. a,b, Glomeruloid vascular proliferation in the stroma of a mucosal melanoma. **a,** H & E; **b,** immunostain for CD34 (streptavidin peroxidase complex technique).

Angiomatoid/pseudoangiosarcomatous pattern

Jain and Allen[58] described endothelioid cells and small pseudovascular lumina in DMM of skin. Adler *et al.*[68] documented a case of metastatic angiomatoid melanoma in the skin which contained large cavernous pseudovascular spaces filled with erythrocytes which initially raised the suspicion of angiosarcoma. The neoplastic cells were, however, S100 protein and HMB-45 positive and yielded negative results with endothelial cell markers. We have observed complex anastomosing pseudo-vascular channels (Figure 10) lined by malignant melanocytic cells in two cases – one in an oral melanoma and the other in a metastatic melanoma in the small intestine. Occasional cellular tufts were also seen projecting within the pseudovascular spaces. These tumours mimicked pseudovascular/pseudoangiosarcomatous squamous cell carcinoma[69,70] and angiosarcoma, respectively.

Associated granulomatous inflammation

Two cases of sarcoid-like epithelioid granulomatous response to metastatic melanomas in the lymph nodes have been documented.[71] In one case small granulomata containing epithelioid histiocytes and Langhans-type giant cells intermingled with melanoma cells whilst in the other well formed sarcoid-like granulomas were seen adjacent to the metastatic tumour deposit. A few cases of tuberculoid granulomas in lymph nodes draining cutaneous melanomas have also been recorded.[72] Rarely, Langerhans cell granulomatosis is seen in such lymph nodes.[73,74]

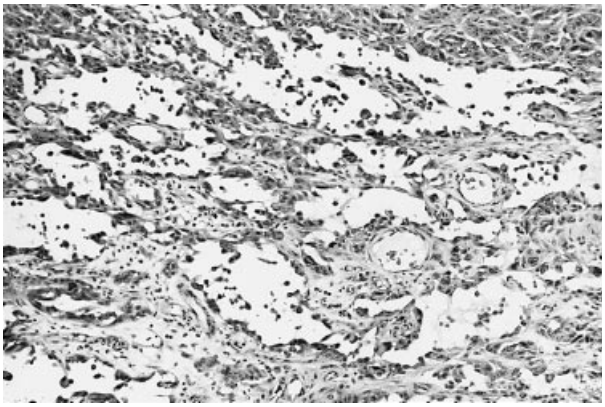


Figure 10. Pseudoangiosarcomatous change in a malignant melanoma (H & E).

Osteoclastic giant cell reaction

Osteoclast-like giant cells are sometimes seen in the stroma of poorly differentiated carcinomas – particularly of breast, pancreas and thyroid. Similar giant cell reaction has been recorded in metastatic melanomas in lymph nodes,[75] bone,[76] soft tissue[15] and lung.[67] In the bone, a melanoma with osteoclast-like giant cells may simulate a primary giant cell tumour. Elsewhere a spindle cell melanoma with osteoclastic giant cells may mimic a giant cell variant of malignant fibrous histiocytoma or a giant cell rich anaplastic carcinoma.

Differentiation towards non-melanocytic elements

As mentioned earlier Schwannian, perineural, fibroblastic and myofibroblastic differentiation is not uncommon in DMM. Rarely melanomas may undergo smooth muscle[77] or rhabdomyoblastic differentiation.[58,78]

A small number of cases of osteocartilaginous differentiation in cutaneous[54,79–84] (particularly subungual) and mucosal melanomas[85,86] have been documented. These tumours may mimic osteo/chondrosarcomas, metaplastic carcinoma or carcinosarcoma. Ganglionic differentiation is an extremely uncommon occurrence[78,79] and recently a case of metastatic melanoma showing ganglioneuroblastic differention[87] has been published. Within the nodal deposit of a metastatic melanoma there were distinct islands of ganglion cells and neuroblasts embedded in a fibrillar stroma. Occasional Homer–Wright rosettes were also present. Interestingly, subsequent metastatic deposits contained only neuroblastic elements and no ganglion cells were seen.

Immunophenotype of melanomas

Malignant melanomas are typically vimentin, NSE, S100 protein,[88–90] NKIC3[91–93] and HMB-45 positive.[90,94–96] Recently anti-Melan-A[97–102]and anti-tyrosinase[103] antibodies have been introduced as two other melanocytic markers (Table 3). Of these, vimentin

Table 3. Immunophenotypes of melanomas

Typical	Aberrant
S100 protein	Cytokeratin
HMB-45	Desmin
NK1C3	Neurofilament protein (NFP)
Melan-A	Glial fibrillary acidic protein (GFAP)
Anti-tyrosinase	α-smooth muscle actin (SMA)
Vimentin	Epithelial membrane antigen (EMA)
Neurone specific enolase	Carcinoembryonic antigen (CEA)
	KP1
	VS38
	Factor XIIIA

and NSE are least useful as they stain a wide variety of tumours. S100 protein and NKIC3 are very useful for screening purposes as both are highly sensitive markers for melanocytic tumours but both lack specificity. Of these two, the S100 protein is more widely used by practising histopathologists. After careful analysis of the published results, Bishop *et al.*[104] found 94% and 95% overall S100 protein positivity for primary and metastatic melanomas, respectively. Usually the morphologically atypical tumours, such as signet-ring cell and rhabdoid melanomas, fail to express this antigen. Fortunately, DMM, a diagnostically difficult group, express this antigen focally or diffusely in almost all cases. NKIC3 is slightly more specific than S100 protein but it also stains many nonmelanocytic tumours such as some carcinomas,[92] neuroendocrine tumours,[92] neurothekeomas,[105] gastrointestinal autonomic neuronal tumours[106] and any tumour in which the cells are rich in lysosomes such as granular cell tumours.[107] HMB-45 is a fairly specific and reliable marker for melanomas and the rate of positivity for conventional primary melanomas varied between 90 and 100% in different studies. The positivity rate drops to 80% in recurrent or metastatic melanomas and spindle cell melanomas are less often positive. The staining in DMM is disappointing as only a few of these tumours express this antigen. For detailed information on HMB-45 the readers are referred to the excellent review article of Bacchi *et al.*[96] Anti-Melan-A (MART-I) monoclonal antibody is a recently discovered melanocytic marker. It stains both benign and malignant melanocytic tumours but, like HMB-45, it fails to label most cases of DMM. The anti-Melan-A antibody, A103, also stains adrenocortical tumours, Leydig cell tumours, Sertoli cell tumours and granulosa cell tumours.[102,108] Tumours of 'perivascular epithelioid cells' (angiomyolipomas, clear-cell sugar tumours and lymphangioleiomyomatosis) which stain with HMB-45, also exhibit positivity for A103 and the other anti-Melan-A antibody M2–7C10.[109] Anti-tyrosinase antibody[103] has also been used as a marker for melanocytic lesions but as far as we are aware, its sensitivity and specificity have not been adequately assessed.

Immunophenotypic aberration

In addition to their protean morphology, melanomas are also known to exhibit immunophenotypic aberrations which may create further diagnostic problems (Table 3). The most frequent of these is the cytokeratin expression.[104,110–113] With the commonly used anticytokeratin antibodies (CAM5.2, AE1/3, etc.) up to 10% of melanomas show positive staining in routinely processed paraffin-embedded material. The positivity is more frequent with CAM5.2 and it is more commonly seen in metastatic tumours. Usually only scattered cells are positive in contrast to the diffuse staining pattern of epithelial tumours. The other epithelial markers which may label melanoma cells are anti-CEA and EMA antibodies.[112–115] The CEA staining is usually seen with polyclonal antibodies and it has been demonstrated in conventional as well as balloon cell melanomas.[34] EMA positivity has been noted rather infrequently and usually focally in conventional melanomas but in one series[62] 43% of DMM exhibited EMA positivity presumably due to perineurial differentiation. In addition to vimentin and cytokeratin, melanoma cells occasionally contain other intermediate filaments. Desmin positivity occurs only rarely in melanomas,[77,116] although Reiman *et al.*[55] found a high rate of desmin positivity (seven of the nine DMM and all 10 conventional melanomas studied in their series were positive), a finding which has not been substantiated by others. NFP positivity has been noted in rare cases using fresh frozen tissue[110] and we have observed occasional NFP-positive cells in a few nasal melanomas and in one case of primary cutaneous small-cell melanoma. We have also noted GFAP positivity in a few cases of DMM which was probably related to the Schwannian differentiation in these tumours. α-SMA positivity is extremely uncommon in conventional cutaneous or metastatic melanomas[104] but many DMM show diffuse positivity for this marker.[62] In our experience, spindle cell nondesmoplastic melanomas usually do not show α-SMA positivity — a feature which helps to differentiate these tumours from leiomyosarcomas. Melanomas may also stain with the so-called plasma cell marker VS38[40] and macrophage marker anti-CD68 (KPI).[45,46,117,118] DMM may stain with the dermal dendrocytic marker Factor XIIIa.[62]

Conclusion

In the review cited in our introduction Arnold Levene[1] concluded with some notes on diagnostic methods. At that time haematoxylin and eosin plus pigment stains were virtually all the histological approaches that were available. Today they remain the bedrock of diagnosis but the advent of electron microscopy and, more importantly, immunohistochemistry have greatly expanded the diagnostic armamentarium. They have clarified some issues and removed some diagnostic uncertainties but at the same time have sewn other seeds of potential confusion. The notoriety of melanoma referred to by Levene persists.

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References

1. Levene A. On the histological diagnosis and prognosis of malignant melanoma. *J. Clin. Pathol*. 1980; **33;** 101–124.
2. Reed WB, Becker SW Sr, Becker SW Jr, Nickel WR. Giant pigmented nevi, melanoma and leptomeningeal melanocytosis. *Arch. Dermatol*. 1965; **91;** 100–119.
3. Hendrickson MR, Ross JC. Neoplasms arising in congenital giant nevi: Morphologic study of seven cases and a review of the literature. *Am. J. Surg. Pathol*. 1981; **5;** 109–135.
4. Nakhleh RE, Wick MR, Rocamora A, Swanson PE, Dehner LP. Morphologic diversity in malignant melanomas. *Am. J. Clin. Pathol*. 1990; **93;** 731–740.
5. Fitzgibbons PL, Martin SE, Simmons TJ. Malignant melanoma metastatic to the ovary. *Am. J. Surg. Pathol*. 1987; **11;** 959–964.
6. Young RH, Scully RE. Malignant melanoma metastatic to the ovary: a clinico-pathologic analysis of 20 cases. *Am. J. Surg. Pathol*. 1991; **15;** 849–860.
7. Franquemont DW, Mills SE. Sinonasal malignant melanoma. A clinicopathologic and immunohistochemical study of 14 cases. *Am. J. Clin. Pathol*. 1991; **96;** 689–697.
8. Attanoos R, Griffiths DFR. Metastatic small cell melanoma to the stomach mimicking primary gastric lymphoma. *Histopathology* 1992; **21;** 173–175.
9. Reed RJ. Giant congenital nevi: a conceptualization of patterns. *J. Invest. Dermatol*. 1993; **10** (Suppl.); 300–312.
10. House NS, Fedok F, Maloney ME, Helm KF. Malignant melanoma with clinical and histologic features of Merkel cell carcinoma. *J. Am. Acad. Dermatol*. 1994; **31;** 839–842.
11. Barnhill RL, Flotte TJ, Fleischli M, Perez-Atayde A. Cutaneous melanoma and atypical Spitz tumors in childhood. *Cancer* 1995; **76;** 1833–1845.
12. Kossard S, Wilkinson B. Nucleolar organizer regions and image analysis nuclear morphometry of small cell (nevoid) melanoma. *J. Cutan. Pathol*. 1995; **22;** 132–136.
13. Barnhill RL. Childhood melanoma. *Sem. Diag. Pathol*. 1998; **15;** 189–194.
14. Regauer S, Anderhuber W, Richtig E, Schachenreiter J, Ott A, Beham A. Primary mucosal melanomas of the nasal cavity and paranasal sinuses. A clinicopathological analysis of 14 cases. *APMIS* 1998; **106;** 403–410.
15. Lodding P, Kindblom L, Angervall L. Metastases of malignant melanoma simulating soft tissue sarcoma. *Virch. Archiv. A. Pathol. Anat*. 1990; **417;** 377–388.
16. Dimaio SM, MacKay B, Smith JL Jr, Dickersin GR. Neurosarcomatous transformation in malignant melanoma: an ultrastructural study. *Cancer* 1982; **50;** 2345–2354.
17. Macak J, Krc I, Elleder M, Lukas Z. Clear cell melanoma of the skin with regressive changes. *Histopathology* 1991; **18;** 276–277.
18. Mourad WA, MacKay B, Ordonez NG, Ro JY, Swanson DA. Clear cell melanoma of the bladder. *Ultrastruct. Pathol*. 1993; **17;** 463–468.
19. Ehara H, Takahashi Y, Saitoh A, Kawada Y, Shimokawa K, Kanemura T. Clear cell melanoma of the renal pelvis presenting as a primary tumour. *J. Urol*. 1997; **157;** 634.
20. Chung EB, Enzinger FM. Malignant melanoma of soft parts: a reassessment of clear cell sarcoma. *Am. J. Surg. Pathol*. 1983; **7;** 405–413.
21. Sheibani K, Battifora H. Signet ring cell melanoma: a rare morphologic variant of malignant melanoma. *Am. J. Surg. Pathol*. 1988; **12;** 28–34.
22. Bonetti F, Colombari R, Zamboni G, Chilosi M, Legnami F. Signet ring melanoma, S100 negative (letter). *Am. J. Surg. Pathol*. 1989; **13;** 522–523.
23. Al-Talib RK, Theaker JM. Signet-ring cell melanoma: light microscopic, immunohistochemical and ultrastructural features. *Histopathology* 1991; **18;** 572–575.
24. Eckert F, Baricevic B, Landthaler M, Schmid U. Metastatic signet-ring cell melanoma in a patient with an unknown primary tumor. *J. Am. Acad. Dermatol*. 1992; **26;** 870–875.
25. Livolsi VA, Brooks JJ, Soslow R, Johnson BL, Elder DE. Signet cell melanocytic lesions. *Mod. Pathol*. 1992; **5;** 515–520.
26. Tsang WYW, Chan JKC, Chow LTC. Signet-ring cell melanoma mimicking adenocarcinoma: a case report. *Acta Cytol*. 1993; **37;** 559–562.
27. Bittesini L, Dei Tos AP, Fletcher CDM. Metastatic malignant melanoma showing a rhabdoid phenotype: further evidence of a non-specific histological pattern. *Histopathology* 1992; **20;** 167–170.
28. Chang ES, Wick MR, Swanson PE, Dehner LP. Metastatic malignant melanoma with 'rhabdoid' features. *Am. J. Clin. Pathol*. 1994; **102;** 426–431.
29. Borek BT, McKee PH, Freeman JA, Maguire B, Brander WL, Calonje E. Primary malignant melanoma with rhabdoid features: a histologic and immunocytochemical study of three cases. *Am. J. Dermatopathol*. 1998; **20;** 123–127.
30. Gardner WA, Vazquez M. Balloon cell melanoma. *Arch. Pathol. Lab. Med*. 1970; **89;** 470–472.
31. Ranchod M. Metastatic melanoma with balloon cell changes. *Cancer* 1972; **30;** 1006–1013.
32. Sondergaard K, Henschel A, Hou-Jensen K. Metastatic melanoma with balloon cell changes: an electron microscopic study. *Ultrastruct. Pathol*. 1980; **1;** 357–360.
33. Peters MS, Su DWP. Balloon cell malignant melanoma. *J. Am. Acad. Dermatol*. 1985; **13;** 351–354.
34. Kao GF, Helwig EB, Graham JH. Balloon cell malignant melanoma of the skin: a clinicopathologic study of 34 cases with histochemical, immunohistochemical and ultrastructural observations. *Cancer* 1992; **69;** 2942–2952.
35. Mowat A, Reid R, MacKie R. Balloon cell metastatic melanoma: an important differential diagnosis of clear cell tumours. *Histopathology* 1994; **24;** 469–472.
36. Al-Nafussi AI, Hughes DE, Williams AR. Hyaline globules in ovarian tumours. *Histopathology* 1993; **23;** 563–566.
37. Severi B, Pasquinelli G, Martinelli GN, Zanetti GF, Santini D. Hyaline globules in ovarian tumours. *Histopathology* 1994; **25;** 299.
38. Monteagudo C, Jorda E, Carda C, Illueca C, Peydro A, Llombart-Bosch A. Erythrophagocytic tumour cells in melanoma and squamous cell carcinoma of the skin. *Histopathology* 1997; **31;** 367–373.
39. Mooi WJ, Krausz T. Cutaneous melanoma. In: *Biopsy Pathology of Melanocytic Disorders*. London: Chapman & Hall, 1992: 283–287.
40. Shanks JH, Banerjee SS. VS38 immunostaining in melanocytic lesions. *J. Clin. Pathol*. 1996; **49;** 205–207.
41. Bhuta S, Mirra JM, Cochran AJ. Myxoid malignant melanoma: a previously undescribed histologic pattern noted in metastatic

lesions and a report of four cases. *Am. J. Surg. Pathol.* 1986; **10;** 203–211.
42. Nottingham JF, Slater DN. Malignant melanoma: a new mimic of colloid adenocarcinoma. *Histopathology* 1988; **13;** 576–578.
43. Garcia-Caballero T, Fraga M, Antunez JR, Perez-Becerra E, Beiras A, Forteza J. Myxoid metastatic melanoma. *Histopathology* 1991; **18;** 371–373.
44. Sarode VR, Joshi K, Ravichandran P, Das R. Myxoid variant of primary cutaneous malignant melanoma. *Histopathology* 1992; **20;** 186–187.
45. Chetty R, Slavin JL, Pitson GA, Dowling JP. Melanoma botryoides: a distinctive myxoid pattern of sino-nasal malignant melanoma. *Histopathology* 1994; **24;** 377–379.
46. McCluggage WG, Shah V, Toner PG. Primary cutaneous myxoid malignant melanoma. *Histopathology* 1996; **28;** 179–182.
47. Collina G, Losi L, Taccagni GL, Mairona A. Myxoid metastases of melanoma: Report of three cases and review of the literature. *Am. J. Dermatopathol.* 1997; **19;** 52–57.
48. Hitchcock MG, White WL. Malicious masquerade: Myxoid melanoma. *Sem. Diagn. Pathol.* 1998; **15;** 195–202.
49. Conley J, Lattes R, Orr W. Desmoplastic melanoma (a rare variant of spindle cell melanoma). *Cancer* 1971; **28;** 914–936.
50. Frolow GR, Shapiro L, Brownstein MH. Desmoplastic malignant melanoma. *Arch. Dermatol.* 1975; **111;** 753–754.
51. Labreque PG, Hu C, Winkelman RK. On the nature of desmoplastic melanoma. *Cancer* 1976; **38;** 1205–1213.
52. Reed RJ, Leonard DD. Neurotropic melanoma (a variant of desmoplastic melanoma). *Am. J. Surg. Pathol.* 1979; **3;** 301–311.
53. From L, Hanna W, Kahn HJ, Gross J, Marks A, Baumal R. Origin of desmoplasia in desmoplastic malignant melanoma. *Hum. Pathol.* 1983; **4;** 1072–1080.
54. Moreno A, Lamarca J, Martinez R, Guix M. Osteoid and bone formation in desmoplastic malignant melanoma. *J. Cut. Pathol.* 1986; **13;** 128–134.
55. Reiman H, Goellner JR, Woods JE, Mixter RC. Desmoplastic melanoma of the head and neck. *Cancer* 1987; **60;** 2269–2274.
56. Kossard S, Doherty E, Murray E. Neurotropic melanoma: a variant of desmoplastic melanoma. *Arch. Dermatol.* 1987; **123;** 907–912.
57. Egbert B, Kempson R, Sagebiel R. Desmoplastic malignant melanoma: a clinicohistopathologic study of 25 cases. *Cancer* 1988; **62;** 2033–2041.
58. Jain S, Allen PW. Desmoplastic malignant melanoma and its variants: a study of 45 cases. *Am. J. Surg. Pathol.* 1989; **13;** 358–373.
59. Bruijn JA, Mihm MC Jr, Barnhill RL. Desmoplastic melanoma. *Histopathology* 1992; **20;** 197–205.
60. Anstey A, McKee P, Wilson-Jones E. Desmoplastic malignant melanoma: a clinicopathologic study of 25 cases. *Br. J. Dermatol.* 1993; **129;** 359–371.
61. Anstey A, Cerio R, Ramnarain N. Desmoplastic malignant melanoma, an immunocytochemical study of 25 cases. *Am. J. Dermatopathol.* 1994; **16;** 14–22.
62. Carlson JA, Dickersin GR, Sober AJ, Barnhill RL. Desmoplastic neurotropic melanoma. *Cancer* 1995; **75;** 478–494.
63. Longacre TA, Egbert BM, Rouse RV. Desmoplastic and spindle cell malignant melanoma. An immunohistochemical study. *Am. J. Surg. Pathol.* 1996; **20;** 1489–1500.
64. Wharton JM, Carlson JA, Mihm MC (Jr). Desmoplastic malignant melanoma: Diagnosis of early clinical lesions. *Hum. Pathol.* 1999; **33;** 537–542.
65. Gaudin PB, Rosai J. Florid vascular proliferation associated with neural and neuroendocrine neoplasms. *Am. J. Surg. Pathol.* 1995; **19;** 642–652.
66. Kepes JJ. Vascular proliferation (letter). *Am. J. Surg. Pathol.* 1996; **20;** 384–386.
67. Suster S, Moran CA. Unusual manifestations of metastatic tumors to the lungs. *Sem. Diagn. Pathol.* 1995; **12;** 193–206.
68. Adler MJ, Beckstead J, White CR. Angiomatoid melanoma: a case of metastatic melanoma mimicking a vascular malignancy. *Am. J. Dermatopathol.* 1997; **19;** 606–609.
69. Banerjee SS, Eyden BP, Wells S, McWilliam LJ, Harris M. Pseudoangiosarcomatous carcinoma: a clinicopathological study of seven cases. *Histopathology* 1992; **21;** 13–23.
70. Nappi O, Wick MR, Pettinato G, Ghiselli RW, Swanson PE. Pseudovascular adenoid squamous cell carcinoma of the skin: a neoplasm that may be mistaken for angiosarcoma. *Am. J. Surg. Pathol.* 1992; **16;** 429–438.
71. Coyne JD, Banerjee SS, Menasce LP, Mene A. Granulomatous lymphadenitis associated with metastatic malignant melanoma. *Histopathology* 1996; **28;** 470–472.
72. de Dulants F, Armijo-Moreno M, Camacho-Martinez F. Tuberculoid granulomata in regional lymph nodes of malignant cutaneous melanoma. *Dermatologica* 1974; **148;** 228–232.
73. Richmond I, Eyden BP, Banerjee SS. Intranodal Langerhans' cell histiocytosis associated with malignant melanoma. *Histopathology* 1995; **26;** 380–382.
74. Roufosse C, Lespagnard L, Sales F, Bron D, Dargent J. Langerhans' cell histiocytosis associated with simultaneous lymphocytic predominance Hodgkin's disease and malignant melanoma (letter). *Hum. Pathol.* 1998; **29;** 200–201.
75. Denton KJ, Stretch J, Athanasou N. Osteoclast-like giant cells in malignant melanoma. *Histopathology* 1992; **20;** 179–181.
76. Daroca PJ, Reed RJ, Martin PC. Metastatic amelanotic melanoma simulating giant cell tumor of bone. *Hum. Pathol.* 1990; **21;** 978–980.
77. Banerjee SS, Bishop PW, Nicholson CM, Eyden BP. Malignant melanoma showing smooth muscle differentiation. *J. Clin. Pathol.* 1996; **49;** 950–951.
78. Slaughter JC, Hardman JM, Kempe LG, Earle KM. Neurocutaneous melanosis and leptomeningeal melanomatosis in children. *Arch. Pathol.* 1969; **88;** 298–304.
79. Wahlstrom T, Saxen L. Malignant skin tumors of neural crest origin. *Cancer* 1976; **38;** 2022–2026.
80. Grunwald MH, Rothem A, Feuerman EJ. Metastatic malignant melanoma with cartilaginous metaplasia. *Dermatologica* 1985; **170;** 249–252.
81. Brisigotti M, Moreno A, Llistosella E, Prat J. Malignant melanoma with osteocartilaginous differentiation. *Surg. Pathol.* 1989; **2;** 73–78.
82. Pellegrini AE, Scalamogna PA. Malignant melanoma with osteoid formation. *Am. J. Dermatopathol.* 1990; **12;** 607–611.
83. Nakagawa H, Imakado S, Nogita T, Ishibahi Y. Osteosarcomatous changes in malignant melanoma: immunohistochemical and ultrastructural studies of a case. *Am. J. Dermatopathol.* 1990; **12;** 162–168.
84. Lucas DR, Tazelaar HD, Unni KK *et al*. Osteogenic melanoma: a rare variant of malignant melanoma. *Am. J. Surg. Pathol.* 1993; **17;** 400–409.
85. Hoorweg JJ, Loftus BM, Hilgers FJM. Osteoid and bone formation in a nasal mucosal melanoma and its metastasis. *Histopathology* 1997; **31;** 465–468.
86. Banerjee SS, Coyne JD, Menasce LP, Lobo CJ, Hirsch PJ. Diagnostic lessons of mucosal melanoma with osteocartilaginous differentiation. *Histopathology* 1998; **33;** 255–260.

87. Banerjee SS, Menasce LP, Eyden BP, Brain AN. Malignant melanoma showing ganglioneuroblastic differentiation: Report of a unique case. *Am. J. Surg. Pathol.* 1999; **23;** 582–588.
88. Cochran AJ, Wen D-R, Herschman HR, Gaynor RB. Detection of S100 protein as an aid to the identification of melanocytic tumors. *Int. J. Cancer* 1982; **30;** 295–297.
89. Wick MR, Swanson PE, De Manivel JC. Immunohistochemical findings in tumors of the skin. In: De Lellis RA, eds. *Advances in Immunohistochemistry.* New York: Raven, 1988: 395–429.
90. Ordonez NG, Xiaolong JI, Hickey RC. Comparison of HMB-45 monoclonal antibody and S100 protein in the immunohistochemical diagnosis of melanoma. *Am. J. Clin. Pathol.* 1988; **90;** 385–390.
91. MacKie RM, Campbell I, Turbitt ML. Use of NKIC3 monoclonal antibody in the assessment of benign and malignant melanocytic lesions. *J. Clin. Pathol.* 1984; **37;** 367–372.
92. Vennegoor C, Calafat J, Hageman Ph *et al.* Biochemical characterization and cellular localization of a formalin – resistant melanoma-associated antigen reacting with monoclonal antibody NKI/C-3. *Int. J. Cancer* 1985; **35;** 287–295.
93. Thomson W, MacKie RM. Comparison of five antimelanoma antibodies for identification of melanocytic cells on tissue sections in rountine dermatopathology. *J. Am. Acad. Dermatol.* 1989; **21;** 1280–1284.
94. Gown AM, Vogel AM, Hoak D, Gough F, McNutt MA. Monoclonal antibodies specific for melanocytic tumors distinguish subpopulations of melanocytes. *Am. J. Pathol.* 1986; **123;** 195–203.
95. Wick MR, Swanson PE, Rocamora A. Rocognition of malignant melanoma by monoclonal antibody HMB-45. An immunohistochemical study of 200 paraffin-embedded cutaneous tumors. *J. Cutan. Pathol.* 1988; **15;** 201–207.
96. Bacchi CE, Bonetti F, Pea M, Martignoni G, Gown AM. HMB-45: a review. *Appl. Immunohistochem.* 1996; **4;** 73–85.
97. Chen YT, Stockert E, Jungbluth A *et al.* Serologic analysis of Melan-A (MART-1), a melanocyte specific protein homogeneously expressed in human melanomas. *Proc. Natl. Acad. Sci.* 1996; **93;** 5915–5919.
98. Fetsch PA, Cormier J, Hijazi YM. Immunocytochemical detection of MART-1 in fresh and paraffin embedded malignant melanomas. *J. Immunother.* 1997; **20;** 60–64.
99. Jungbluth AA, Busam KJ, Gerald WL *et al.* A103: an anti-melan A monoclonal antibody for the detection of malignant melanoma in paraffin-embedded tissues. *Am. J. Surg. Pathol.* 1998; **22;** 595–602.
100. Busam KJ, Chen Y-T, Old LJ *et al.* Expression of Melan-A (MART-1) in benign melanocytic naevi and primary cutaneous malignant melanoma. *Am. J. Surg. Pathol.* 1998; **22;** 976–982.
101. Blessing K, Sanders DSA, Grant JJH. Comparison of immunohistochemical staining of the novel antibody Melan-A with S100 protein and HMB-45 in malignant melanoma and melanoma variants. *Histopathology* 1998; **32;** 139–146.
102. Busam KJ, Jungbluth AA. Melan-A, a new melanocytic differentiation marker. *Adv. Anat. Pathol.* 1999; **6;** 12–18.
103. Takimoto H, Suzuki S, Masui S *et al.* MAT-1, a monoclonal antibody that specifically recognizes human tyrosinase. *J. Invest. Dermatol.* 1995; **105;** 764–768.
104. Bishop PW, Menasce LP, Yates AJ, Win NA, Banerjee SS. An immunophenotypic survey of malignant melanomas. *Histopathology* 1993; **23;** 159–166.
105. Calonje E, Wilson-Jones E, Smith NP, Fletcher CDM. Cellular 'neurothekeoma': an epithelioid variant of pilar leiomyoma? Morphological and immunohistochemical analysis of a series. *Histopathology* 1992; **20;** 397–404.
106. Shanks JH, Harris M, Banerjee SS, Eyden BP. Gastrointestinal autonomic nerve tumours: a report of nine cases. *Histopathology* 1996; **29;** 111–121.
107. Buley ID, Gatter KC, Kelly PMA, Heryet A, Millard PR. Granular cell tumours revisited. An immunohistological and ultrastructural study. *Histopathology* 1988; **12;** 263–274.
108. Busam KJ, Iversen K, Coplan KA *et al.* Immunoreactivity for A103, an antibody to Melan-A (MART-1), in adrenocortical and other steroid tumors. *Am. J. Surg. Pathol.* 1998; **22;** 57–63.
109. Fetsch PA, Fetsch JF, Marincola FM, Travis W, Batts KP, Abati A. Comparison of melanoma antigen recognized by T cells (MART-1) to HMB-45: additional evidence to support a common lineage for angiomyolipoma, lymphangiomyomatosis, and clear cell sugar tumor. *Mod. Pathol.* 1998; **11;** 699–703.
110. Miettinen M, Franssila K. Immunohistochemical spectrum of malignant melanoma: the common presence of keratins. *Lab. Invest.* 1989; **61;** 623–628.
111. Zarbo RJ, Gown AM, Nagle RB, Visscher DW, Crissman JD. Anomalous cytokeratin expression in malignant melanoma: one-and two-dimensional western blot analysis and immunohistochemical survey of 100 melanomas. *Mod. Pathol.* 1990; **3;** 494–501.
112. Ben-Izhak O, Stark P, Levy R, Bergman R, Lichtig C. Epithelial markers in malignant melanoma. A study of primary lesions and their metastases. *Am. J. Dermatopathol.* 1994; **16;** 241–246.
113. Ben-Izhak O, Levy R, Weill S *et al.* Anorectal malignant melanoma. A clinicopathologic study, including immunohistochemistry and DNA flow cytometry. *Cancer* 1997; **79;** 18–25.
114. Selby WL, Nance KV, Park HK. CEA immunoreactivity in metastatic malignant melanoma. *Mod. Pathol.* 1992; **5;** 415–419.
115. Sanders DS, Evans AT, Allen CA *et al.* Classification of CEA-related positivity in primary and metastatic malignant melanoma. *J. Pathol.* 1994; **172;** 343–348.
116. Truong LD, Rangdaeng S, Cagle P, Ro JY, Hawkins H, Font RL. The diagnostic utility of desmin: a study of 584 cases and review of the literature. *Am. J. Clin. Pathol.* 1990; **93;** 305–314.
117. Facchetti F, Bertalot G, Grigolato PG. KP1 (CD68) staining of malignant melanomas. *Histopathology* 1991; **19;** 141–145.
118. Gloghini A, Rizzo A, Zanette I *et al.* KP1/CD68 expression in malignant neoplasms including lymphomas, sarcomas and carcinomas. *Am. J. Clin. Pathol.* 1995; **103;** 425–431.