DP-3
Immunohistochemical staining of BerEP4 and epithelial membrane antigen in sebaceoma is distinct from basal cell carcinoma

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Sebaceoma is a benign adnexal skin tumour composed of at least 50% basaloid (germinative) cells and a smaller proportion of mature sebocytes. Sebaceomas with sparse mature sebocytes may closely resemble basal cell carcinoma (BCC). Rarely bona fide BCC may display focal sebaceous differentiation. Correct diagnosis is important since BCC is a locally invasive malignant tumour. Sebaceoma is benign but may be a marker for Muir-Torré syndrome. BerEP4 is useful in differentiating adenocarcinomas (positive) from mesothelioma (negative). In normal skin BerEP4 stains strongly in anagen buds of hair follicles and sweat gland coils but is absent in germinative (basaloid) cells of sebaceous glands. BerEP4 is strongly expressed in all BCC subtypes whereas epithelial membrane antigen (EMA) is only focally expressed in keratotic and squamoid areas of keratotic and basosquamous BCC (Tellechea O, Reis JP, Domingues JC, Baptista AP. Monoclonal antibody BerEP4 distinguishes basal cell carcinoma from squamous cell carcinoma of the skin. *Am J Dermatopathol* 1993;15:452–5) (Beer TW, Shepherd P, Theaker JM. BerEP4 and epithelial membrane antigen aid distinction of basal cell, squamous cell and basosquamous carcinomas of the skin. *Histopathology* 2000;37:218-23). We aimed to describe the BerEP4 and EMA immunohistochemical staining pattern in sebaceoma and highlight its potential utility in differentiating sebaceoma from BCC. We reviewed 25 cases of sebaceoma, diagnosed in the last 5 years, from 23 patients (age range 40–94 years) and examined haematoxylin and eosin, BerEP4 and EMA stained sections in all cases. The histological features of sebaceoma were confirmed in all cases. Three cases showed focal peripheral palisading and retraction artefact, more usually considered characteristic of BCC. Two cases had sparse mature sebocytes, histologically resembling BCC. Twenty-four (95%) of 25 cases were negative for BerEP4. A single case exhibited focal BerEP4 staining in <10% tumour cells, predominantly mature sebocytes. EMA staining was generally absent in germinative cells but was strongly expressed in approximately 50% (range 20–80%) of mature sebocytes, highlighting their cytoplasmic vacuoles. In summary, absent or minimal BerEP4 staining distinguishes sebaceoma, including the subset displaying only minimal sebaceous differentiation, from BCC. EMA staining of cytoplasmic vacuoles is typical of sebaceous differentiation. Sebaceoma and BCC are considered to arise from germinative cells in the folliculo-sebaceous unit. The BerEP4 staining profile provides evidence of derivation from anatomically and antigenically distinct compartments, i.e. sebaceous and follicular respectively.

DP-4
Spitz tumours reveal distinctive kinetics, VEGF-C and microvessel profiles by topographic compartments

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The bases of the cell kinetics and microvessel profiles in Spitz tumours (ST) are poorly understood. No study has correlated cell kinetics, microvessel profile, and VEGF-C expression by topographic compartments in ST to date. We selected 42 ST, 42 malignant melanomas (MM) of which 15 were in the radial growth phase (RGP) and 27 in the vertical growth phase (VGP), and 35 conventional melanocytic naevi (15 junctional, 20 compound); the latter two groups were used as controls. Immunostaining for Ki-67 and vascular endothelial growth factor-C (VEGF-C), and *in situ* end labelling (ISEL) of DNA fragments (using the Klenow fragment of DNA polymerase I) were scored according to topographic compartments: junctional, superficial dermal (above 0·76 mm) and deep dermal (below 0·76 mm), screening the whole compartment in each case. Appropriate controls were run in each sample. CD-31-stained slides were used to estimate microvessel density. The results were compared statistically using analysis of variance and Student t-test, and considered significantly different if $P < 0{\cdot}05$. A superficial-to-deep gradient was maintained for Ki-67 in all lesions, but was significantly higher in MM. From junctional to deep dermal compartments, ST showed a progressive and statistically significant increase of ISEL indices (4·38%, 4·73%, 8·35%) and microvessel density (4·38, 4·39, 7·41 vessels HPF^{-1}). VEGF-C expression by compartments appears in the Table 1. In conclusion, the local VEGF-C expression in ST is directly correlated with the microvessel density and apoptosis index and inversely correlated with the proliferation index, suggesting that this distinctive blood vessel pattern is reactive to regressive cellular changes rather than an element of melanocytic lesion progression.

Table 1. Vascular endothelial growth factor C expression

	Junctional	Superficial Dermis	Deep Dermis
Junctional Naevi	14·92	–	–
Compound Naevi	9·25	2·38	1·47
MM-RGP	30·68	14·05	19·25
MM-VGP	58·93	32·53	33·00
ST	54·34	46·65	49·45

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DP-1
BerEP4 and EMA staining in basaloid and squamous skin tumours: the potential pitfall of positive BerEP4 staining in basaloid variant of Bowen's disease

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Squamous cell carcinoma (SCC) and bowenoid actinic keratosis (Bowen's) showing basaloid morphology may mimic basal cell carcinoma (BCC), particularly in small and crushed biopsies. Previous groups have reported the utility of BerEP4 and epithelial membrane antigen (EMA) in this regard (Tope WD, Nowfar-Rad M, Kist DA. BerEP4-positive phenotype differentiated actinic keratosis (AK) from superficial BCC. *Dermatol Surg* 2000;26:415–8) (Beer TW, Shepherd P, Theaker JM. BerEP4 and epithelial membrane antigen aid distinction of basal cell, squamous cell and basosquamous carcinomas of the skin. *Histopathology* 2000;37:218-23). We have used the panel BerEP4 and EMA in over 400 cases from 2001 to 2006. In this retrospective study we describe staining patterns in 85 cases of selected basaloid or squamous tumours in which a confident diagnosis was possible on the original haematoxylin and eosin sections. Our findings confirm the consistent staining of BerEP4 in all BCC subtypes, EMA staining only focally (≤10%) in keratotic and squamoid areas. Bowen's disease with basaloid morphology commonly (58% of cases) showed moderate or strong BerEP4 staining, often in at least 50% of tumour. EMA staining was moderate or strong in at least 20% of the tumour in these cases. In contrast, BerEP4 rarely showed significant staining in nonbasaloid AK or Bowen's. Most basaloid SCC in our small series showed a similar immunophenotype to other SCC. The frequent strong expression of BerEP4 in a basaloid variant of Bowen's is a potential diagnostic pitfall, which has not been previously reported. However, the presence of at least 20% moderate to strong EMA staining helps distinguish these morphologically difficult tumours from BCC. The staining pattern in basaloid SCC needs further study.

The staining findings are tabulated below:

Diagnosis (number of cases)	BerEP4		EMA	
	≥ ++**	≥ 50%***	≥ ++ **	≥ 50%***
Basaloid* Bowen's (19)	10	8	19	12
AK / Bowen's (21)	1	1	12	5
Basaloid* SCC (5)	1	1	4	3
SCC (9)	1	0	8	7
BCC: Basosquamous (11)	11	11	3	0
BCC: Others (20)	20	17	4	0

* Basaloid morphology in at least 50% of tumour

** Cases with tumour showing areas of moderate or strong staining

*** Cases with >50% of tumour showing moderate or strong staining

DP-2
Deep apoptosis down-regulation is the kinetic hallmark of cutaneous Merkel cell carcinomas

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Merkel cell carcinomas (MCCs) are unusual cutaneous neuroendocrine neoplasms with high cellular turnover and heterogeneous histology (large to small cell type, sometimes associated with differentiated components, in particular, squamous cell carcinoma). Although the knowledge of cell kinetics in these neoplasms can help us to design better therapeutic protocols, no detailed analysis of cell kinetics (proliferation and apoptosis) by topographic compartments has been available to date. MCCs included in this series were required to express at least one epithelial and two neural markers from a panel including the cytokeratin cocktail AE1–AE3, cytokeratin 20, synaptophysin, chromogranin A, neurofilament protein, and neuron-specific enolase, along with consistent ultrastructural findings. We selected 21 MCCs of the small cell type with no differentiated component to evaluate mitotic figure (MF) counting, Ki-67 index, and apoptosis index based on the *in situ* end labelling (ISEL) of fragmented DNA using digoxigenin-labelled dUTP and *Escherichia coli* DNA polymerase I (Klenow fragment). At least 50 high-power fields were screened per topographic compartment (superficial or papillary dermis, and deep or reticular dermis), recording the average and standard deviation for each variable. Variables were statistically compared in each tumour compartment using analysis of variance and Student *t*-test (significant if $P < 0{\cdot}05$ in two-tailed distributions), and the correlation coefficient calculated for proliferation-apoptosis in each compartment. MCCs revealed high cell density (over 425 cell HPF^{-1}) and no statistical differences for the proliferation markers by topographic compartments (superficial $10{\cdot}10 \pm 1{\cdot}99\%$, deep $10{\cdot}90 \pm 2{\cdot}53\%$). Apoptosis showed significantly lower values in the deep compartment (superficial $1{\cdot}84 \pm 0{\cdot}80\%$, deep $1{\cdot}16 \pm 0{\cdot}62\%$, $P = 0{\cdot}005$). Proliferation and apoptosis were correlated in the superficial compartment only ($R^2 = 0{\cdot}89$). In conclusion, homogeneously distributed high proliferation defines MCCs. Apoptosis follows the distribution pattern of proliferation in superficial compartments, whereas in deep compartments apoptosis is down-regulated, is less variable and is independent of proliferation. These features should be considered when designing any effective treatment.