Immunohistochemical Features of Merkel Cell Carcinoma in Correlation with Presence of Merkel Cell Polyomavirus DNA

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Merkel cell carcinoma (MCC) is a neuroendocrine malignant neoplasm that primarily affects sun-exposed skin of older Caucasian and/or immunosuppressed persons (1, 2). Its biological behaviour is highly aggressive, with high rates of metastasis and poor survival (1).

Merkel cell polyomavirus (MCPyV) was identified in January 2008 by Feng et al. (3) in tumour tissue from MCC patients, proving clonal integration of the virus DNA into the host genome. Meanwhile, several studies confirmed this observation, showing frequent prevalence of MCPyV DNA in MCCs (2, 4, 5), suggesting MCPyV as the likely causative agent of MCC. Presence of MCPyV DNA in MCC seems to be a relevant favourable prognostic factor (5) and MCCs potentially have different invasion and metastatic properties depending on their MCPyV DNA status.

This is the first report analysing CK20, CK19, CD117 and ST3 protein expression of tumour cells as a function of presence of MCPyV DNA in a large cohort of MCC.

METHODS

Thirty-four MCC samples from 30 patients were analysed in an earlier study for the presence of MCPyV DNA by PCR and Southern-blot hybridization of PCR products, resulting in 22 cases of MCPyV DNA-positive MCCs and 12 cases of MCPyV DNA-negative MCCs (2). All MCC specimens were stained with CK19, CK20, CD117 and ST3. For each tumour sample, staining intensity and percentage of positive tumour cells (PP) for CK20, CK19, CD117 and ST3 were semi-quantitatively evaluated by two investigators (MJF and CA) with excellent overall concordance using the grading system described by Remmele & Stegner (6). Statistical calculation was performed with Fisher's exact test, comparing differences in immunohistochemistry dependent on MCPyV DNA status of MCCs. $p < 0.05$ displays statistical significance.

Table I. *Association of CK20, CK19, CD117 and ST3 expression with Merkel cell polyomavirus (MCPyV) DNA prevalence on Merkel cell carcinomas*

	MCPyV DNA					
	Positive *n* (%)	Total (%)	Negative *n* (%)	Total (%)	All (%)	*p*-value[a]
n=34 samples	*22*	*64.7*	*12*	*35.3*	*100*	0.353
CK20 positive	22 (100)	64.7	11 (91.7)	32.3	97	
CK20 negative	0	0	1 (8.3)	3	3	
n=32 samples	*20*	*62.5*	*12*	*37.5*	*100*	0.249
CK19 positive	5 (25)	15.6	6 (50)	18.8	34.4	
CK19 negative	15 (75)	46.9	6 (50)	18.7	65.6	
n=30 samples	*20*	*66.7*	*10*	*33.3*	*100*	0.461
CD117 positive	7 (35.0)	23.3	5 (16.7)	16.7	40	
CD117 negative	13 (65.0)	43.3	5 (16.7)	16.7	60	
n=30 samples	*20*	*66.7*	*10*	*33.3*	*100*	0.709
ST3 positive	10 (50)	33.3	4 (40)	13.3	46.7	
ST3 negative	10 (50)	33.3	6 (60)	20.0	53.3	

[a]Fisher's exact test.

RESULTS AND DISCUSSION

In a previous study we confirmed data from Feng et al. (3) proving frequent prevalence of MCPyV DNA in MCCs (21 of 33; 64%). Furthermore, our clinical investigations are in line with Sihto et al. (5), showing that MCPyV DNA-positive MCCs tend to be preferentially located on the limbs and tend to metastasize less frequently (7). These observations imply a different biological behaviour of MCCs, dependent on their MCPyV DNA status, eventually resulting in varying protein expression patterns of tumour cells.

Anti-cytokeratin 20 (CK20)-staining is concordant with data from the previous literature showing a "paranuclear dot-like pattern" in 97% of all included MCCs (1). This highly sensitive staining-feature is very important for routine histopathology to distinguish MCCs from other small round blue cell tumours (8, 9). Independently of MCPyV DNA status, CK20 was expressed in nearly all MCCs (Table I; $p = 0.353$). Only one case in our cohort did not stain for CK20, but was positive for CK19 in the typical "paranuclear dot-like pattern" thus establishing the diagnosis. CK19, the smallest human keratin, is expressed in undifferentiated germinative basaloid cells and is usually not expressed by cells of non-epithelial origin (10). In healthy skin CK19 is expressed in secretory sweat glands and ductal cells and in the bulge region of the outer root sheath of the hair follicle. We found CK19-expression in 11 of our 32 specimens, interestingly twice as much in MCPyV DNA-negative MCCs compared with MCPyV DNA-positive, displaying an additional helpful marker in CK20-negative MCCs (50% vs. 25%, Table I; $p = 0.249$). The proto-oncogene *c-kit* encodes a transmembrane receptor *CD117/c-kit* protein. CD117 is a transmembrane protein of the receptor tyrosine kinase family that is important for haematopoiesis, gametogenesis, melanogenesis and the development of interstitial cells of Cajal (11). This antibody, which is found in haematopoeitic stem cells, melanocytes and mast cells, has been reported in MCCs, but not in physiological Merkel cells. Su et al. (12) found presence of CD117 in 13 of 16 (81%) MCCs and concluded an early event in Merkel cell transformation. However, only 12 of 30 MCCs (40%)

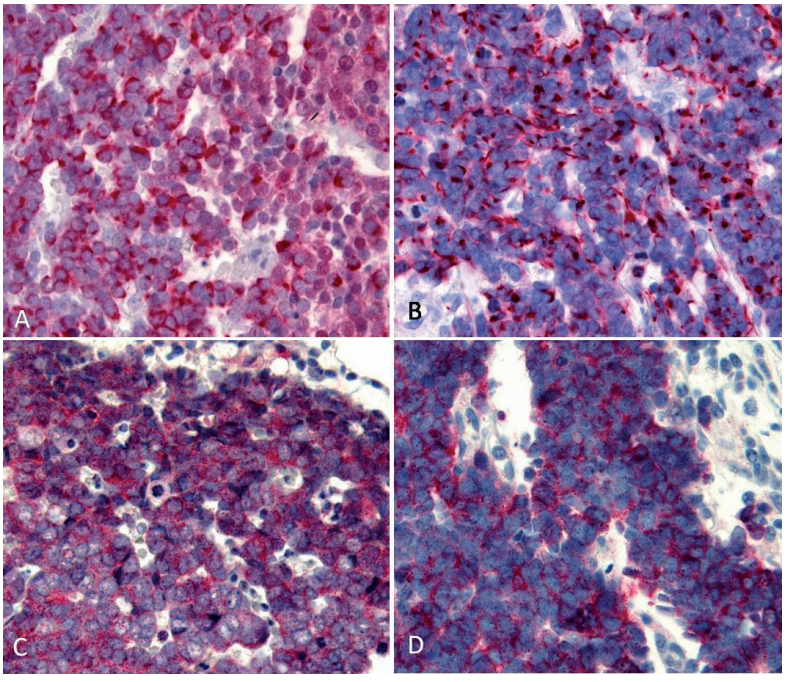

Fig. 1. Positive immunoreaction in Merkel cell carcinoma for cytokeratin 19 (A, ×400), cytokeratin 20 (B, ×400), stromelysin-3/MMP-11 (C, ×400) and CD117/c-kit (D, ×400). Presented staining intensity is strong (staining intensity=3) in every case.

in our cohort stained positive for CD117, but this was more than twice as frequent (35.0% vs. 16.7%, Table I, $p=0.461$) in MCPyV DNA-positive MCCs compared with MCPyV DNA-negative MCCs.

Stromelysin-3/matrix metalloproteinase11 (ST3), a member of the matrix metalloproteinase family, over-expression is associated with tumour invasion and poor prognosis in numerous carcinomas (13). On the other hand, ST3 is also known to be expressed in benign dermatofibromata and absent in locally aggressive and invasive dermatofibrosarcoma protuberans (14, 15). Review of the literature shows that ST3 is an active partner of cancer cells along the whole natural cancer history, and is essential for optimal tumour development, as it reduces the death of cancer cells invading adjacent connective tissues at the primary tumour site (13). Paradoxically, ST3 lowers metastasis development *in vivo* in mice. However, this beneficial effect does not compensate the deleterious anti-apoptotic function of ST3 (13). Interestingly, we found ST3 expression of MCC tumour cells in 14 of 30 specimens (46.7%). We assume that ST3 expression may be beneficial for tumour invasion; however, there is no statistical significant correlation with MCPyV DNA-presence ($p=0.709$).

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The authors declare no conflicts of interest.

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