Published in final edited form as:

J Dermatol Sci. 2012 July ; 67(1): 66–68. doi:10.1016/j.jdermsci.2012.03.006.

Lack of integrin $\beta 5$ in Merkel cell carcinomas and derived cell lines is frequently associated with Merkel cell polyomavirus positivity

Baki Akgül¹, Paola Zigrino², Martin Hufbauer¹, Xi Liu³, Patrick S. Moore³, Cornelia Mauch², and Herbert Pfister¹

¹Institute of Virology, National Reference Centre for Papilloma- and Polyomaviruses, University of Cologne, Germany

²Department for Dermatology and Venerology, University of Cologne, Germany

³Cancer Virology Program, University of Pittsburgh Cancer Institute, Pittsburgh, USA

To the editor:

Merkel cell carcinoma (MCC) is a rare but aggressive neuroendocrine skin neoplasia. The merkel cell polyomavirus (MCPyV), a double-stranded DNA tumor virus was discovered in 2008. It is found in approximately 80% of human MCC and is suspected to play a role in MCC development [1]. Both, small tumor and the aminoterminal moiety of the large tumor antigen, were found to be expressed in MCPyV-positive MCC [2]. Merkel cells are usually scattered in the basal cell layer of the epidermis along the dermo-epidermal junction [3].

A number of MCC cell lines, with and without clonally integrated MCPyV, have been established from MCC tumours and represent valuable tools to study aspects of tumourigenesis *in vitro*. The MCPyV-negative cell lines UIISO [4], MCC13 [5] and MCC26 [6] grow adhesively, whereas MaTi cells [7] grow in suspension. The MCPyV-positive cells MKL-1, MS-1, WaGa [2] and MKL-2 [6] all grow in suspension. Cells growing in suspension have not only lost their capability to bind to the extracellular matrix (ECM), but also proliferate slower than those growing adherently [2, 7].

Cells of all types interact with the ECM via integrins, heterodimeric transmembrane receptors composed by an α and β subunit. Integrins not only participate in cell-ECM adhesion, but may also function as receptors that transduce signals to the cell interior via the cytoskeleton and thus stimulate proliferation [8]. We therefore hypothesized that non-adherent MCC cell lines may show differences in integrin expression compared to adherent MCC cell lines, which are responsible for attachment and their growth behaviour. To prove this, we evaluated the expression of integrin subunits $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, αv , $\beta 1$, $\beta 3$, $\beta 4$, $\beta 5$ and $\beta 6$, known to be expressed in the human skin [8], on the adherent and non-adherent MCC cell lines. Samples were analyzed in duplicate together with a dilution series of a standard-plasmid containing the corresponding cDNA, which was used to generate a standard curve. To emphasize the relative differences between subunits, the mRNA expression levels were calculated relative to the absolute amounts of Hypoxanthin-Phosphoribosyl-Transferase-1 (HPRT1) transcripts used as internal control. The expression of integrin subunits was initially measured in the adherent MCPyV-negative line UIISO and

Correspondence: Jun.-Prof. Dr. Baki Akgül, Institute of Virology, University of Cologne, Fürst-Pückler-Str. 56, 50935 Cologne, Germany, Tel.: ++49 221 478 3911, Fax: ++49 221 478 3904, baki.akguel@web.de.

Conflict of interest: The authors state no conflict of interest.

the MCPyV-positive line MS-1, growing in suspension. The qRT-PCR results revealed that both cell lines do not express integrin $\alpha 2$, $\alpha 5$, $\alpha 6$, $\beta 3$, $\beta 4$ and $\beta 6$ (data not shown). The integrins αv and $\beta 1$ are expressed in comparable amounts by both UIISO and MS-1 cells whereas transcript levels of integrin $\alpha 3$ and $\beta 5$ were high in UIISO cells and barely detectable in MS-1 cells, with a 96- and 2180-fold difference respectively (Figure 1a). To confirm this observation at the protein level Western blots of cell extracts were evaluated. In line with qRT-PCR results, $\alpha 3$ and $\beta 5$ proteins were highly expressed in UIISO cells but undetectable in MS-1 cells (Figure 1b).

We next studied the expression of $\alpha 3$ - and $\beta 5$ -integrin levels on additional MCPyV-positive and MCPyV-negative cell lines using whole cell lysates. As shown in Figure 1c, the expression level of $\beta 5$ -integrin was also high in the adherent MCPyV-negative cell lines MCC13 and MCC26 but low in MaTi cells, which grow in suspension. In the MCPyV-positive lines MKL-1, MKL-2 and WaGa, all growing in suspension, $\beta 5$ -integrin was undetectable. In all these cell lines, $\alpha 3$ -integrin could not be detected. The expression of $\alpha 3$ -integrin in UIISO cells thus appears to be exceptional and lack of $\beta 5$ -integrin expression correlates with the MCC cell line growth in suspension. This does not exclude that other transmembrane molecules contribute to cell adhesion.

This phenotype is also rather strongly correlated with MCPyV positivity (Table S1). We were next interested to study $\beta 5$ -integrin expression in MCPyV-positive and -negative MCC *in vivo* and therefore performed immunohistochemical staining on paraffin embedded sections. Nine MCC cases were obtained from the files of the Department of Dermatology and Venerology of the University of Cologne. MCPyV load quantification has been performed as previously described [9]. For virus-negative MCC, three showed strong (Fig. 2A) and two showed intermediate $\beta 5$ -integrin staining. For MCPyV-positive MCC, one tumor with a load of 2.6 viral DNA copies per cell had intermediate $\beta 5$ -integrin staining (Fig. 2B) while the remaining three tumors (one with a viral load of 2.8 MCPyV DNA copies per cell and two with 23 copies per cell) showed weak staining for $\beta 5$ -integrin (Fig. 2C). Thus, viral positivity inversely correlated with $\beta 5$ -integrin expression.

This data provides some explanation, why MCC cell lines show distinct growth behaviour in culture related to MCPyV status. *In vivo* MCPyV-positive MCC are more likely to have low $\beta 5$ -integrin, which is characteristic for MCC cell lines growing in suspension. In adherent cells the presence of $\beta 5$ and αv subunits can lead to formation of the vitronectin receptor $\alpha v\beta 5$, which may be important for attachment and migration of Merkel cells. Changes in cell-matrix adhesion may affect metastasis formation. Whether, in addition to viral positivity a progressive loss of $\beta 5$ -integrin expression influences MCC prognosis is presently not clear. Additional studies are needed to clarify, if MCPyV directly or indirectly affects $\beta 5$ -integrin expression and thereby growth and attachment of Merkel cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the German National Reference Center for Papilloma- and Polyomaviruses (German Federal Ministry of Health, grant number FKZ 1369-401) and US NCI grants CA136363 and CA120726. We thank Masahiro Shuda, Huichen Feng and Yuan Chang, University of Pittsburgh, for advice and help with obtaining MCC cell lines.

References

1. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science*. 2008; 319:1096–1100. [PubMed: 18202256]
2. Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, Moore PS, et al. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc Natl Acad Sci U S A*. 2008; 105:16272–16277. [PubMed: 18812503]
3. Halata Z, Grim M, Bauman KI. Friedrich Sigmund Merkel and his "Merkel cell", morphology, development, and physiology: review and new results. *Anat Rec A Discov Mol Cell Evol Biol*. 2003; 271:225–239. [PubMed: 12552639]
4. Ronan SG, Green AD, Shilkaitis A, Huang TS, Das Gupta TK. Merkel cell carcinoma: in vitro and in vivo characteristics of a new cell line. *J Am Acad Dermatol*. 1993; 29:715–722. [PubMed: 8227544]
5. Leonard JH, Dash P, Holland P, Kearsley JH, Bell JR. Characterisation of four Merkel cell carcinoma adherent cell lines. *Int J Cancer*. 1995; 60:100–107. [PubMed: 7814141]
6. Van Gele M, Leonard JH, Van Roy N, Van Limbergen H, Van Belle S, Cocquyt V, et al. Combined karyotyping, CGH and M-FISH analysis allows detailed characterization of unidentified chromosomal rearrangements in Merkel cell carcinoma. *Int J Cancer*. 2002; 101:137–145. [PubMed: 12209990]
7. Houben R, Shuda M, Weinkam R, Schrama D, Feng H, Chang Y, et al. Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. *J Virol*. 2010; 84:7064–7072. [PubMed: 20444890]
8. Janes SM, Watt FM. New roles for integrins in squamous-cell carcinoma. *Nat Rev Cancer*. 2006; 6:175–183. [PubMed: 16498442]
9. Wieland U, Silling S, Scola N, Potthoff A, Gambichler T, Brockmeyer NH, et al. Merkel cell polyomavirus infection in HIV-positive men. *Arch Dermatol*. 2011; 147:401–406. [PubMed: 21482890]

Figure 1.

(A) Integrin mRNA expression in UIISO and MS-1 cells was quantified in qRT-PCR and normalized to the expression level of HPRT1. (B, C) Total cell extracts were prepared with RIPA buffer and analysed by Western blotting. Blots were probed with antibodies to tubulin (clone YL1/2, Abcam), $\beta 5$ -integrin (rabbit polyclonal Abcam, Cambridge, UK) and $\alpha 3$ -integrin (clone c-18, Santa Cruz, Heidelberg, Germany).

Figure 2. Formalin fixed and paraffin-embedded MCC sections were stained for $\beta 5$ -integrin and counterstained with hematoxylin. MCC with high (A), medium (B) and weak (C) $\beta 5$ -integrin staining are shown. Magnification: 200x.