CD3 monoclonal antibodies to eleven *P. acnes*-positive acne biopsies [early stage, comedone ($n = 3$) and later stage, inflamed lesion ($n = 8$)] and to nine *P. acnes*-negative acne biopsies to ascertain if CD3+ cells can be located in the vicinity of hair follicles harbouring *P. acnes*. Clusters of CD3+ cells could be seen in the vicinity of the *P. acnes*-positive comedones (Fig. 2E and F). This is in line with earlier reports that elevated numbers of CD3+ and CD4+ T cells were detected in the perifollicular and papillary dermis around microcomedones, and that the initial cellular infiltrate in early inflammatory acne lesions is mononuclear, predominantly CD4+ T cells [8]. Therefore, co-localization of *P. acnes* and CD3+ lymphocytes could be considered indicative of a possible role for *P. acnes* in the initiation of inflammation. While clusters of CD3+ could be seen in the biopsies containing the inflamed acne lesions, these cells have been virtually lacking in the vicinity of the *P. acnes*-positive inflamed lesions (Fig. 2G and H). This observation suggests a limited role for the cellular arm of the immune system in the pathogenesis of *P. acnes*-positive inflamed lesions. A previous study has shown that TLR2+, CD3+ and CD14+ cells are present in the inflammatory infiltrate around the perifollicular/peribulbar regions in acne lesions [9]. However, it is not known if these acne lesions were positive for *P. acnes*. Biofilms are known for their ability to resist attack by the host immune system [10]. Why *P. acnes* biofilms in the early non-inflammatory stage of acne are accompanied by a cellular response, while those in the later, inflammatory stage, are not, is currently unclear.

In conclusion, we have clearly demonstrated that, in the majority of *P. acnes* positive acne patients, hair follicles are colonised by *P. acnes* macrocolonies/biofilms and that inflammation in *P. acnes*-positive inflamed lesions is not directly linked to cellular immune response.

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Appendix A. Supplementary data

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Letter to the Editor

Lack of integrin β5 in Merkel cell carcinomas and derived cell lines is frequently associated with Merkel cell polyomavirus positivity

To the Editor,

Merkel cell carcinoma (MCC) is a rare but aggressive neuroendocrine skin neoplasia. The Merkel cell polyomavirus (MCPyV), a double-stranded DNA tumour 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 tumour and the aminoterminal moiety of the large tumour 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 UISO [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 α2, α3, α5, α6, αv, β1, β3, β4, β5 and β6, known to be expressed in the human skin [8], on the adherent and non-adherent MCC cell lines. Samples were analysed 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 UISO and the MCPyV-positive line MS-1, growing in suspension. The qRT-PCR results revealed that both cell lines do not express integrin α2, α5, α6, β3, β4 and β6 (data not shown). The integrins αv and β1 are expressed in comparable amounts by both UISO and MS-1 cells whereas transcript levels of integrin α3 and β5 were high in UISO cells and barely detectable in MS-1 cells, with a 96- and 2180-fold difference respectively (Fig. 1A). To confirm this observation at the protein level Western blots of cell extracts were evaluated. In line with qRT-PCR results, α3 and β5 proteins were highly expressed in UISO cells but undetectable in MS-1 cells (Fig. 1B).

We next studied the expression of α3- and β5-integrin levels on additional MCPyV-positive and MCPyV-negative cell lines using whole cell lysates. As shown in Fig. 1C, the expression level of β5-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, β5-integrin was undetectable. In all these cell lines, α3-integrin could not be detected. The expression of α3-integrin in UISO cells thus appears to be exceptional and lack of β5-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 β5-integrin expression in MCPyV-positive and -negative MCC *in vivo* and therefore performed immunhistochemical 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 β5-integrin staining. For MCPyV-positive MCC, one tumour with a load of 2.6 viral DNA copies per cell had intermediate β5-integrin staining (Fig. 2B) while the remaining three tumours (one with a viral load

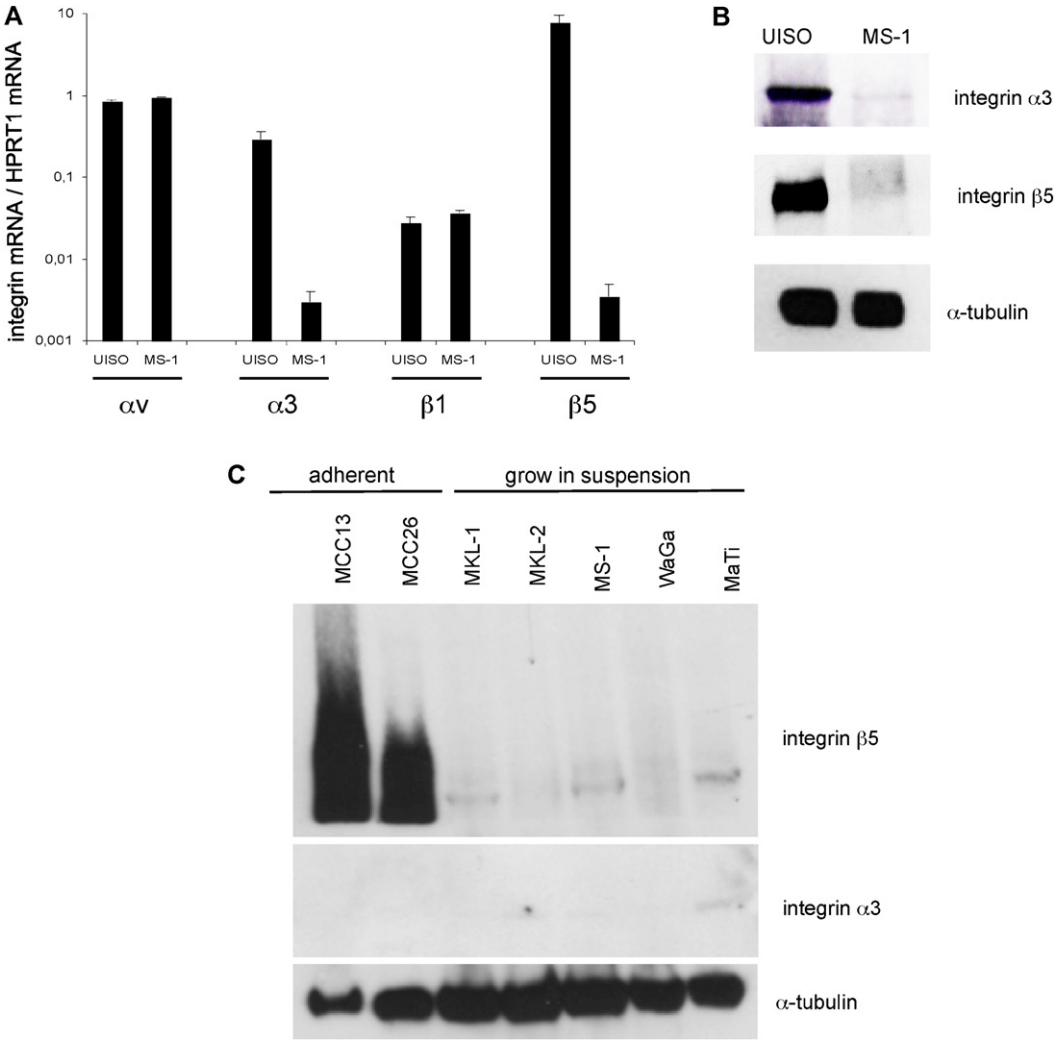

Fig. 1. (A) Integrin mRNA expression in UISO and MS-1 cells was quantified in qRT-PCR and normalized to the expression level of HPRT1. (B and C) Total cell extracts were prepared with RIPA buffer and analysed by Western blotting. Blots were probed with antibodies to tubulin (clone YL1/2, Abcam), β5-integrin (rabbit polyclonal Abcam, Cambridge, UK) and α3-integrin (clone c-18, Santa Cruz, Heidelberg, Germany).

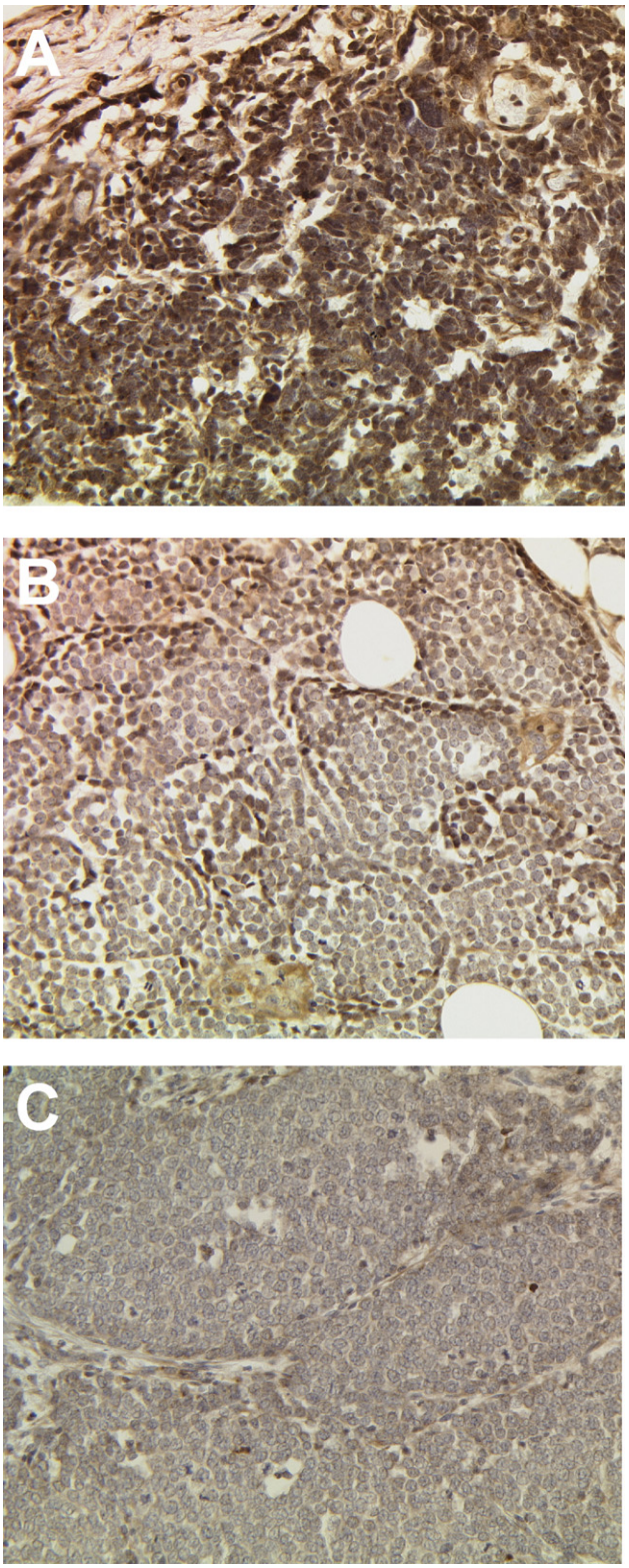

Fig. 2. Formalin fixed and paraffin-embedded MCC sections were stained for β5-integrin and counterstained with haematoxylin. MCC with high (A), medium (B) and weak (C) β5-integrin staining are shown. Magnification: 200×.

of 2.8 MCPyV DNA copies per cell and two with 23 copies per cell) showed weak staining for β5-integrin (Fig. 2C). Thus, viral positivity inversely correlated with β5-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 β5-integrin, which is characteristic for MCC cell lines growing in suspension. In adherent cells the presence of β5 and αv subunits can lead to formation of the vitronectin receptor αvβ5, 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 β5-integrin expression influences MCC prognosis is presently not clear. Additional studies are needed to clarify, if MCPyV directly or indirectly affects β5-integrin expression and thereby growth and attachment of Merkel cells.

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Appendix A. Supplementary data

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