of DP contained ~40–60% of either very weak or DiI-negative cells within each DP. These data raise the possibility of recruitment of some mouse cells to a portion of the reconstituted DP and still remains to be investigated. DiI-positive cells were also found in the neo-dermis that formed in the implantation, which is consistent with the recent reports of Qiao *et al.* (2008, 2009).

The morphology and size of hair follicles induced by human DP spheres resembled the ones induced by mouse dermal cells. This was in contrast to our expectation that human scalp DP cells would produce larger hairs than those obtained with mouse dermal cells. However, our data are in line with the finding that pelage-type hair follicle is induced by patch assay despite DP spheres being made using vibrissa DP cells (Osada *et al.*, 2007). Our data, together with those of Osada *et al.* (2007), suggest that the size of DP sphere, which shrank during cultivation, may account for the small size of regenerated hair follicles.

Higgins *et al.* (2010) very recently reported that 3D human DP spheres prepared by hanging-drop cultures have expression profiles different from papilla cells cultured in 2D but with many similarities to intact DPs; these findings may account for our successful hair induction using human DP spheres. In conclusion, using a reconstitution assay, we show that sphere formation increases the ability of cultured human DP cells to induce hair follicles from mouse epidermal cells, which to our knowledge is previously unreported.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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No Evidence for Association of HPyV6 or HPyV7 with Different Skin Cancers

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TO THE EDITOR

It has long been suspected that polyomaviruses might contribute to the carcinogenesis of human cancers. Indeed, viral proteins expressed by human polyomaviruses are known to initiate transformation and immortalization of cultured cells and can cause cancer in experimentally challenged animals (reviewed in Abend *et al.* (2009) and Eisenberg *et al.* (2009)). Nevertheless, compelling direct evidence for the impact of polyomaviruses on human cancers was missing until 2008, when Feng *et al.* (2008) demonstrated the clonal integration of a new human polyomavirus in most Merkel cell carcinomas (MCC). Subsequent reports from various labs worldwide have confirmed the presence of this Merkel cell polyomavirus (MCV) in about 85% of MCC

Abbreviations: HPyV, human polyomavirus; MCC, Merkel cell carcinomas; MCV, Merkel cell polyomavirus

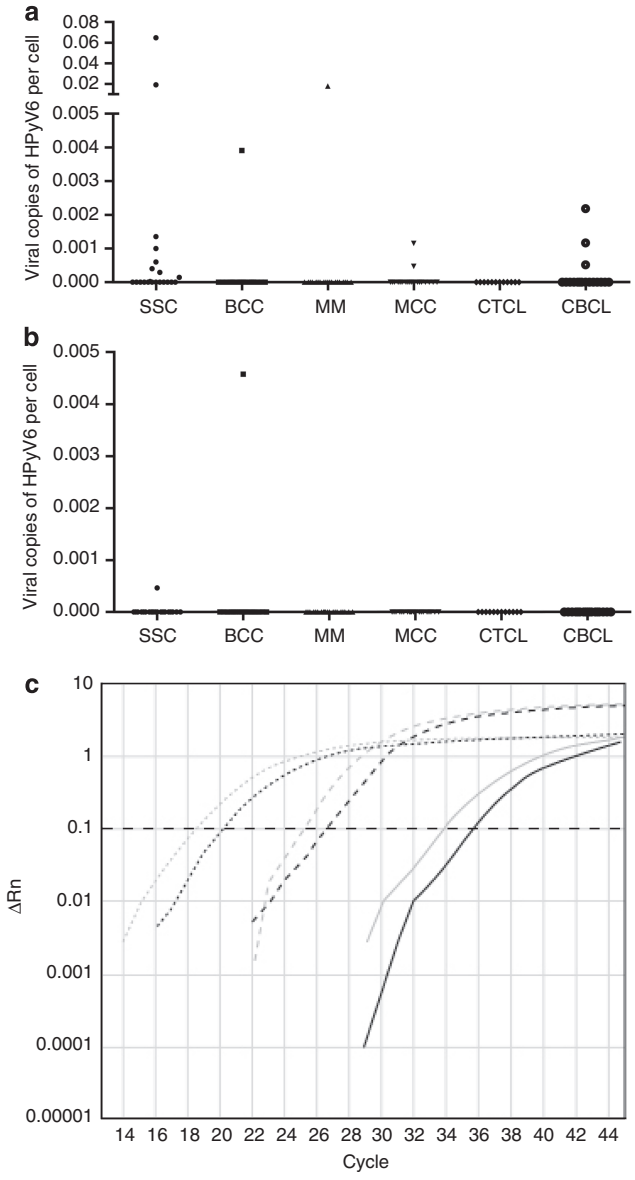

Figure 1. Detection of low levels of HPyV6 and HPyV7 in skin cancer. Viral load of (**a**) HPyV6 and (**b**) HPyV7 was calculated using the $\Delta\Delta C_t$ method and a calibrator consisting of one viral genome/human genome. The samples were normalized to cellular long interspersed nuclear element-1 (LINE-1) control. (**c**) Depiction of real-time PCR data for two different MCC samples (gray vs. black). Cellular DNA controls LINE-1 and thyroid peroxidase are shown as dotted and dashed lines, respectively. HPyV6 is shown as a solid line. BCC, basal cell carcinoma; CBCL, cutaneous B-cell lymphoma; CTCL, cutaneous T-cell lymphoma; HpyV, human polyomavirus; MCC, Merkel cell carcinoma; MM, melanoma; SCC, squamous cell carcinoma.

cases (Kassem *et al.*, 2008; Becker *et al.*, 2009). A causal role of MCV for most MCCs is sustained by our recent observation of oncogenic addiction of MCV-positive MCC cell lines to the expression of MCV-encoded T antigens (Houben *et al.*, 2010). In fact, the interaction of large T antigen with retinoblastoma protein is important for promoting growth of MCV-positive MCC cells (Houben *et al.*, 2011).

Besides MCV, four new human polyomaviruses have been identified in the last year. One virus was found associated with a rare skin condition called trichodysplasia spinulosa (van der Meijden *et al.*, 2010), while human polyomavirus 9 (HPyV9) was discovered in blood samples from immunosuppressed subjects (Scuda *et al.*, 2011). Two other viruses, HPyV6 and HPyV7, were detected in skin swabs of healthy persons (Schowalter *et al.*, 2010). HPyV6 and HPyV7 DNA were detected in swabs from 5 and 4 of the 35 healthy individuals, respectively. Moreover, a pilot serological study demonstrated seropositivity of 69% for HPyV6 and 35% for HPyV7 in 95 blood donors, indicating that infections with these viruses are very common. Thus, similar to MCV, HPyV6 and HPyV7 seem to be constituents of the human skin microbiome. These observations prompted us to test whether HPyV6 or HPyV7 might have an impact on skin cancer, just as MCV has on MCC. To this end, DNA from formalin-fixed tumor samples was analyzed for the presence of HPyV by TaqMan technology. All selected samples consisted of at least 50% of tumor cells. The used primers (HpyV6_forward: 5′-CCAGGTAGTGAT GCATTGAAACTT-3′; HpyV7_forward: 5′-AATAGCAGTCAAAGCACCAGCAT-3′; HpyV6_reverse: 5′-TCGTCCCAGCCTC TATTGAAA-3′; HpyV7_reverse: 5′-CC TGCTGTTGTATTCATTGCATTT-3′) and probes (HPyV6_TP: Vic-CCACCTCCAC AATATGGCAGTCCCG-BHQ; HPyV7_TP: Fam-CCGGTGGTCTTTAGCATACTCTT TGGCC-BHQ) were specific for the respective large T-antigen genes. PCR efficiencies determined with plasmids containing the appropriate viral genome were very similar for both viruses, i.e., 96.8% for HPyV6 and 94.6% for HPyV7. A total of 108 samples consisting of 21 squamous cell carcinoma (SCC), 18 basal cell carcinoma, 20 melanoma, 20 MCV-negative MCC, 12 cutaneous T-cell lymphomas, and 17 cutaneous B-cell lymphomas were analyzed. Estimations of the viral load, i.e., copies of viral genome/human genome, of HPyV6 or HPyV7 in the individual samples were done by the $\Delta\Delta C_t$ method; the repetitive element long interspersed nuclear element served as endogeneous control for normalization and a 1:1 ratio of human genome equivalents and plasmids carrying the respective viral genome was used as calibrator. In the analyzed series of skin cancers, HPyV6 was more often detectable than HPyV7; indeed, HPyV6 was traceable in 15 (14%) and HPyV7 only in 2 (2%) samples (Figure 1a and b). Only one sample, an SCC, contained both viruses. Owing to sequence

divergences, such as polymorphisms in the primer-binding regions, our present study might underestimate the frequencies detectable in the skin cancer samples. Nevertheless, the copy number of the viruses per human genome within the positive tested samples was very low. In the representative examples depicted in Figure 1c, HPyV6 showed much higher cycle thresholds than the cellular gene control *thyroid peroxidase*, which is normally unaffected by gains and losses in MCC and thus should represent a curve for two copies per cell (Paulson *et al.*, 2009). The more frequent presence of HPyV6 compared with HPyV7 among the skin tumor samples is in line with the serological data available for these viruses (Schowalter *et al.*, 2010). The observations of a similar detection rate of HPyV6 and HPyV7 on the skin surface of healthy volunteers, the higher seropositivity for HPyV6, and an increased presence of HPyV6 in skin tumor samples are suggestive for either a broader cell tropism, a higher survival capability within the skin, or a more efficient skin entry mechanism of HPyV6. The low viral load presence of HPyV6 and HPyV7 in some of the cancer samples reflects the situation of MCV, which can also be detected at low quantities in different tissue specimens irrespective of the pathological status (Loyo *et al.*, 2010). In summary, the low level presence of HPyV6 and HPyV7 within the 108 analyzed skin cancer samples, i.e., clearly lower than one viral copy per cell, does not deliver any evidence for a significant role of these polyomaviruses for the pathology of the respective skin cancers, but it certainly exclude a role of these viruses in the maintenance of the malignant phenotype of the analyzed skin cancers.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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GM-CSF-Independent CD1a Expression in Epidermal Langerhans Cells: Evidence from Human *CD1A* Genome-Transgenic Mice

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TO THE EDITOR

Epidermal Langerhans cells (LCs) are central in various aspects of skin biology, but the fact that the cells are the sole cell type constitutively expressing high levels of CD1a molecules has been appreciated only when researchers use CD1a as a specific marker of human LCs. Although we (Sugita *et al.*, 1999; Pena-Cruz *et al.*, 2003) and others (Hunger *et al.*, 2004; de Jong *et al.*, 2010) recently reported studies focusing on the functional role of CD1a in lipid antigen presentation and T-cell activation in the skin, a fundamental question remains to be addressed as to how such high levels of CD1a expression are maintained in LCs. Certain factors and stimuli have been implicated in CD1a expression in myelomonocytic cells, among which GM-CSF is the most important because of its ability to induce CD1a transcription and translation, as well as differentiation into LC-like cells