Direct cellular reprogramming enables development of viral T antigen–driven Merkel cell carcinoma in mice

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Merkel cell carcinoma (MCC) is an aggressive neuroendocrine skin cancer that frequently carries an integrated Merkel cell polyomavirus (MCPyV) genome and expresses viral transforming antigens (TAgs). MCC tumor cells also express signature genes detected in skin-resident, postmitotic Merkel cells, including atonal bHLH transcription factor 1 (ATOH1), which is required for Merkel cell development from epidermal progenitors. We now report the use of in vivo cellular reprogramming, using ATOH1, to drive MCC development from murine epidermis. We generated mice that conditionally expressed MCPyV TAg and ATOH1 in epidermal cells, yielding microscopic collections of proliferating MCC-like cells arising from hair follicles. Immunostaining of these nascent tumors revealed p53 accumulation and apoptosis, and targeted deletion of transformation related protein 53 (Trp53) led to development of gross skin tumors with classic MCC histology and marker expression. Global transcriptome analysis confirmed the close similarity of mouse and human MCCs, and hierarchical clustering showed conserved upregulation of signature genes. Our data establish that expression of MCPyV TAg in ATOH1-reprogrammed epidermal cells and their neuroendocrine progeny initiates hair follicle–derived MCC tumorigenesis in adult mice. Moreover, progression to full-blown MCC in this model requires loss of p53, mimicking the functional inhibition of p53 reported in human MCPyV-positive MCCs.

Introduction

Merkel cell carcinoma (MCC) is an aggressive neuroendocrine skin tumor with a poor prognosis (1), although up to 50% of patients with advanced disease respond to immunotherapy (2). In 2008, a clonally integrated polyomavirus was discovered in 8 of 10 MCC tumors (3). Subsequent studies confirmed that most MCC tumors contain the integrated Merkel cell polyomavirus (MCPyV) genome and express 2 viral transforming antigens (TAgs), small T (sTAg) and truncated large T (tLTAg) (4, 5). MCPyV-negative MCCs have a high mutation burden with a predominance of UV signature mutations, whereas relatively few mutations are detected in MCPyV-positive MCCs (6–9), arguing that viral TAg play a central role in virus-positive MCC tumorigenesis. In keeping with this notion, MCPyV TAg transform cultured cells (10, 11) and are tumorigenic when expressed in vivo (12–16), but a bona fide mouse model of MCC has not been reported despite over a decade of effort by several laboratories.

MCC tumor cells express multiple transcription factors and lineage markers in common with Merkel cells, which are rare, non-proliferative neuroendocrine cells that reside beneath specialized compartments of epidermal cells and transduce light touch and itch sensation to adjacent sensory nerves (17, 18). The cell of origin of MCC is not known (11, 16), hindering efforts to develop a viable mouse model testing the role of MCPyV TAg in MCC development. However, normal Merkel cells arise from KRT5+ epidermal progenitors through the action of atonal bHLH transcription factor 1 (ATOH1) (19, 20), and some human MCCs are closely associated with epidermal tumors (21–24), raising the possibility of a common cellular origin. Moreover, ectopic ATOH1 expression can reprogram epidermal cells to form postmitotic Merkel cells in adult mice (25), and we have previously shown that expression of ATOH1 together with MCPyV sTAg yields MCC-like cells in mouse embryos (15). Given the failure of conventional approaches to generate a mouse model of MCC, we set out to ascertain whether ATOH1 could be utilized as a tool to reprogram TAg-expressing epidermal cells into the Merkel cell lineage in adult mice, enabling the development of murine tumors resembling human MCC.

Conflict of Interest: The authors have declared that no conflict of interests exist.

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SLA mice 2.5 weeks or more after transgene induction revealed that LTAg expression became largely restricted to hair follicle epithelium (Supplemental Figure 2). Moreover, histological analysis of sections from SLA mice collected 2 weeks after transgene induction revealed spatially restricted, atypical-appearing cellular aggregates near the normally quiescent hair follicle stem cell compartment called the bulge (refs. 29, 30, and Figure 1). The cells in these aggregates contained scant cytoplasm, condensed chromosomes, and pyknotic nuclei (Figure 1B), and they expressed ATOH1, the Merkel cell/MCC markers keratin 8 (KRT8) and SOX2, proliferation marker Ki67, apoptosis marker cleaved caspase-3 (CC3), MCPyV tLTAg, and p53 (Figure 1C), none of which were detectable at appreciable levels in control hair follicles (Supplemental Figure 3). While a reliable sTAg antibody is not available, the sTAg target REST corepressor 2 (RCOR2) (31) was also detected in the atypical cellular aggregates (Supplemental Figure 4A). Although microscopic cellular aggregates with KRT8+ cells could be detected at all time points examined between 2 weeks and 12 months after transgene induction, they were not detected in control hair follicles.

Results and Discussion

We generated and validated transgenic mouse strains with doxycycline-inducible coexpression of MCPyV sTAg and tLTAg carrying internal ribosome entry site–driven (IRES-driven) red fluorescent protein (RFP) and GFP reporters, respectively (Figure 1A and Supplemental Figure 1; supplemental material available online with this article https://doi.org/10.1172/JCI152069DS1). We next performed crosses with K5-CreERT2 (26), R26-LSL-rtTA (27), and tetO-Atoh1 mice (28) to generate K5-CreERT2;R26-LSL-rtTA;tetO-sT/tLT;tetO-Atoh1 (SLA) mice, which we designated SLA mice, as well as SL mice, which were missing the tetO-Atoh1 allele (see Methods). Mice were treated with tamoxifen to activate Cre function and rtTA expression and with doxycycline to induce expression of sTAg and tLTAg, with or without ATOH1, in Krt5-expressing epidermal cells and their progeny.

Although the K5-CreERT2 strain drives recombination broadly in the basal layer of hair follicles as well as the interfollicular epidermis (26), examination of tissue sections from TAg-expressing SL mice 2.5 weeks or more after transgene induction revealed that LTAg expression became largely restricted to hair follicle epithelium (Supplemental Figure 2). Moreover, histological analysis of sections from SLA mice collected 2 weeks after transgene induction revealed spatially restricted, atypical-appearing cellular aggregates near the normally quiescent hair follicle stem cell compartment called the bulge (refs. 29, 30, and Figure 1). The cells in these aggregates contained scant cytoplasm, condensed chromosomes, and pyknotic nuclei (Figure 1B), and they expressed ATOH1, the Merkel cell/MCC markers keratin 8 (KRT8) and SOX2, proliferation marker Ki67, apoptosis marker cleaved caspase-3 (CC3), MCPyV tLTAg, and p53 (Figure 1C), none of which were detectable at appreciable levels in control hair follicles (Supplemental Figure 3). While a reliable sTAg antibody is not available, the sTAg target REST corepressor 2 (RCOR2) (31) was also detected in the atypical cellular aggregates (Supplemental Figure 4A). Although microscopic cellular aggregates with KRT8+ cells could be detected at all time points examined between 2 weeks and 12 months after transgene induction, they were not detected in control hair follicles.

Figure 1. In vivo reprogramming using ATOH1 enables initiation of murine MCC development in mice. (A) Combination of mouse strains used to generate SLA mice, expressing MCPyV sTAg, tLTAg, and ATOH1, in Krt5-expressing cells and their progeny. (B) Nascent tumors arising from hair follicle epithelium in SLA mice. Scale bars: 50 μm. (C) Immunostaining for the indicated markers. Scale bars: 25 μm.
fourteen SLAP mice were euthanized for humane reasons, reflecting unanticipated morbidity also reported in other mouse models expressing MCPyV TAgs and deficient in p53 (12, 13). Each of the remaining 8 mice developed 1 or more grossly visible tumors resembling human MCCs between 11 and 22 weeks after transgene induction (Figure 2, B and C). Notably, the WT Trp53 allele was lost in all MCCs for which DNA was available for analysis (n = 5) (Supplemental Methods and Supplemental Figure 5, showing absence of sequence from amplicons covering Trp53 exons 2-10), pointing to a requirement for complete loss of p53 for tumor expansion.

Ten of the eleven skin tumors arising in SLAP mice exhibited histologic features highly characteristic of human MCCs, including a monomorphous small blue cell phenotype, finely stippled chromatin, prominent mitoses, and nuclear molding (Figure 2C) (see Methods). Variable numbers of tumor cells also expressed tLTAg and ATOH1 (Figure 2D) as well as multiple protein mark-
from both species based on an average Spearman’s correlation of 0.74. In addition, the scatter plot in Supplemental Figure 7 shows concordance of the most highly upregulated genes in MCCs of both species when compared with normal skin, and Supplemental Figure 8 shows pathway enrichment analysis comparing mouse MCCs to normal skin. Finally, examination of transcripts highly expressed in mouse Merkel cells (ref. 35 and Figure 3B) or neuroendocrine variants of lung, prostate, and bladder cancers (ref. 36 and Supplemental Figure 9) highlighted the molecular similarities of mouse and human MCCs to normal Merkel cells as well as neuroendocrine cancers arising in other organs, respectively.

Although multiple polyomaviruses infect humans (37), only MCPyV has been convincingly linked to a human cancer. Using direct in vivo cellular reprogramming with ATOH1, we have generated what we believe is the first adult murine model of MCC. Several of our findings are noteworthy. Despite the broad expression pattern of the Krt5 promoter in skin epithelia, initiation of TAg-driven mouse MCCs in our model appears to occur in or near a restricted domain of the hair follicle that harbors several stem cell populations (30). This is of interest since MCPyV-positive human MCCs have a low burden of UV mutations, in keeping with a cell of origin that resides in deeper compartments of skin, including the hair follicle, rather than more superficial regions, such as the interfollicular epidermis (see Figure 1B). In addition, the hair follicle is a site of relative immune privilege (38), perhaps allowing for survival and expansion of viral antigen-expressing cells that may be eliminated if recognized as foreign in other regions of skin. Finally, stem or progenitor cells may have greater plasticity and thus be preferentially susceptible to ATOH1-mediated postnatal reprogramming into the Merkel cell lineage.

The requirement for Trp53 deletion in our model is also of interest since TP53 mutations are uncommon in MCPyV-positive human MCCs, perhaps because p53 is depleted due to sTAg-mediated upregulation of MDM2 and the MDM4 activator CK1α (32). However, efficient disruption of p53 function seems unlikely in our murine SLA model, given the accumulation of p53 in nascent tumors (Figure 1C). The differential requirement for loss of Trp53 in viral TAg-driven mouse MCC, but not human MCC, may also be due to the striking divergence of p53-regulated tar-
get genes in mouse versus human cells (39). Our data argue that either functional inactivation of p53 in human MCCs or genetic deletion of Trp53 in our mouse model is required for MCPyV-driven MCC tumorigenesis. Importantly, our immunostaining studies and transcriptomic data highlight the strong similarity between human and mouse MCCs despite the different mechanisms leading to inhibition of p53.

In summary, our findings underscore the utility of modulating cell fate to generate a neoplasm without a defined cell of origin; establish a pivotal role for MCPyV T antigens in the pathogenesis of virus-positive MCCs; demonstrate how tumors that appear to reside entirely within the dermis may originate from follicle epithelia; and set the stage for future studies centered on gaining deeper insight into MCC biology, mechanisms underlying viral TAg-driven tumorigenesis, and preclinical testing of novel therapeutics.

Methods

Mouse models

Transgenic mouse production. Transgenic mice carrying doxycycline-inducible MCPyV T antigens and fluorescent reporters, designated tetO-sT/tLT, were generated by coinjection of tetO-sTA1-ires-egfp (tetO-sT) and tetO-lTAg-ires-gfp (tetO-lT) cassettes (Supplemental Figure 1A) into fertilized (C57BL/6 × SJL) F2 mouse oocytes by the University of Michigan Transgenic Animal Model Core. Details and characterization of tetO-sT/tLT transgenic mice are provided in Supplemental Methods and Supplemental Figure 1.

Generating mice with inducible transgene expression. To generate a model that would allow tight control of transgene expression both spatially and temporally, we employed a triple-transgenic model that included (a) a hormone-inducible Cre allele, (b) a Cre-inducible rTA allele, and (c) tetO-driven effector alleles. We employed the K5-CreERT2 strain (26) to drive tamoxifen-inducible Cre activity in K5-expressing epidermal cells, including Merkel cell progenitors; B6.Cg-Gt(Rosa)26Sor+ /+ /tetO-Lox51EGFP + / + /J mice (The Jackson Laboratory, stock no. 005670; ref. 27), designated R26-LSL-rT A, to drive expression of rTA in recombined cells and all of their progeny; and tetO-sT/tLT strains for expression of MCPyV s TAg and lTAg in mice with a target genotype of K5-CreERT2;R26-LSL-rTA; tetO-Lox51/Lox51 (SL). A tetO-Atoh1 allele (28) was added to drive cells into the neuroendocrine lineage in K5-CreERT2;R26-LSL-rTA;tetO-sT/Lox51 (SLA) mice. In mice treated with tamoxifen to activate Cre function, recombination at the ROSA locus leads to rTA expression, and transgene expression is induced by doxycycline. To also alter Trp53 gene dosage, mice were crossed with conditional B6.129P2-Trp53fl/fl/J (Trp53fl/fl) mutant mice (The Jackson Laboratory, stock no. 008462; ref. 33) to generate K5-CreERT2;R26-LSL-rTA;tetO-Lox51/Lox51;Trp53fl/fl (SLAP) mice, enabling deletion of 1 copy of the Trp53 gene after recombination. Additional details are provided in Supplemental Methods and Supplemental Table 1.

Transgene induction and tumor monitoring. Transgene expression with or without Trp53 deletion was induced in SLA (n = 15; 11F / 4M) and SLAP (n = 14; 10F / 4M) mice starting at P21-P24 by continuous administration of tamoxifen chow at 400 mg/kg in Teklad Global rodent diet (Envigo) and doxycycline (200 μg/ml, Fisher Scientific) in drinking water containing 5% sucrose. Mice were monitored biweekly for skin phenotypes and tumor development. SLA mice were moni-
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