Are there Multiple Cells of Origin of Merkel Cell Carcinoma?

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Abstract

Merkel cell carcinoma (MCC) is a rare but lethal cancer with the highest case-by-case fatality rate among all skin cancers. 80% of cancers are associated with the Merkel cell polyomavirus (MCPyV). 20% of MCCs are virus negative. Recent epidemiological data suggest that there are important, clinically relevant differences between these two subtypes of MCC. Recent studies in cancer genomics, mouse genetics, and virology experiments have transformed our understanding of MCC pathophysiology. Importantly, dramatic differences in the genetics of these two MCC subtypes suggest fundamental differences in their pathophysiology. We review these recent works and find that they provocatively suggest that MCPyV-positive and MCPyV-negative MCCs arise from two different cells of origin: the MCPyV-negative MCC from epidermal keratinocytes and the MCPyV-positive MCC from dermal fibroblasts. If true, this would represent the first cancer that we are aware of that evolves from cells of origin from two distinct germ layers: MCPyV-negative MCCs from ectodermal keratinocytes and MCPyV-positive MCCs from mesodermal fibroblasts. Future epigenetic experiments may prove valuable in confirming these distinct lineages for these MCC subtypes, especially for the clinical importance the cell of origin has on MCC treatment and prevention.

Introduction

Merkel Cell Carcinoma (MCC) is a highly aggressive primary skin cancer with a case-by-case fatality rate worse than stage-matched melanomas. It usually presents as a rapidly growing pink-red dome-shaped nodule with a strong preference for sun-exposed areas.¹ Histologically, MCC are small blue cell tumors tightly packed into sheets or trabecular arrays that express neuroendocrine markers with scant cytoplasm.² The name for MCCs comes from the structural and immunohistochemical (IHC) characteristics they share with Merkel cells. However, there has been substantial debate in the literature about the cells of
origin of MCC, with various groups suggesting that MCCs do not arise from Merkel cells but instead arise from epidermal stem cells, dermal stem cells, or pre/pro-B cells.

Recently, there have been dramatic advances in our understanding of the molecular underpinnings of MCC. In 2008, Chang, Moore, and colleagues discovered that ~80% of MCCs are associated with the Merkel Cell Polyomavirus (MCPyV). The virus appears to be cancer promoting. In MCPyV-positive MCCs but not in other cell types, the MCPyV virus is clonally integrated into the host genome. These MCPyV-positive MCCs durably express viral oncoproteins that are normally transiently expressed in the viral life cycle.

The MCPyV genome has diverged from other human polyomaviruses but still encodes for the oncoproteins Large T (LT) and Small T (ST) antigens. While the LT antigen of polyomaviruses binds to and represses both RB and p53, MCPyV LT specifically inhibits RB function but cannot bind p53. In contrast to ST antigens from other polyomaviruses, the MCPyV ST antigen does not require binding to the protein phosphatase 2a protein to promote carcinogenesis. Instead, its oncogenic activity derives from a protein domain that binds to and inhibits multiple E3 ligation proteins including FBW7. Underscoring the oncogenic properties of MCPyV ST and LT antigens, downregulation of LT or ST expression impairs the viability and growth of MCPyV-positive MCC. Importantly, however, in ~20% of MCCs, there is no evidence of clonal viral integration or expression of viral oncoproteins, suggesting that these tumors are MCPyV-negative.

Recent epidemiological data suggest that there are clinically relevant differences between these two populations. The highest worldwide incidence of MCCs is found in Australia, a country with a predominantly Caucasian population with high year round UV exposure. Notably, as compared to the USA and elsewhere where ~80% of MCC are MCPyV-positive, the MCC burden in Australia is substantially skewed towards virus negative tumors. In addition, MCPyV-negative tumors are less frequently found on extremities and more frequently found in the head and neck as compared to MCPyV-positive tumors. Importantly, this difference appears to be clinically significant, as MCPyV-negative tumors appear to be more aggressive with increased risk of progression (HR 1.77) and death (HR = 1.85) due to MCC. This highlights the need to understand the distinct pathophysiology of MCPyV-negative and MCPyV-positive MCCs.

Cancer is fundamentally a genetic disease. To advance our understanding of the genetic bases of MCC, we and others recently performed exome sequencing of MCCs MCPyV-positive and MCPyV-negative MCCs. These efforts provided novel insights into the distinct pathogeneses of virus-positive and virus-negative MCCs and led us to revisit important fundamental questions about MCC biology including its cell(s) of origin.

The number and nature of somatic mutations in cancer are a function of cell-intrinsic and environmental factors. Multiple groups have shown that mutational analysis can enable lineage tracing to follow cells as they evolve during health and disease. For example, mutational signatures can be used to infer the environmental exposures to potential carcinogens. This has been used in cancer to track the life histories of tumors, inferring the stem cell origin of cancer, and tracking tumor evolution during tumor metastasis.
acquired drug resistance.\textsuperscript{25} We utilize these data, in combination with other recent discoveries, to posit that virus-positive and virus-negative MCCs have different cells of origin from two different germ layers.

**Distinct life histories of virus-positive and virus-negative MCC**

Exome sequencing of MCCs has provided important insights into the pathophysiology and targetability of the disease.\textsuperscript{19–21} Notably, these sequencing efforts confirm the low prevalence of potentially targetable mutations, particularly in the PI-3-kinase pathway in MCCs. In addition, they provide the genetic explanation for the immunogenicity of MCPyV-negative MCCs, namely the high incidence of tumor neoantigens.\textsuperscript{19–21}

Strikingly, we and others observed that the mutational burden in MCCs is bimodal.\textsuperscript{19–21} One cohort has a relatively low mutation burden, 0.4–0.75 mutations/Mb, which is lower than that of any epithelial cancers sequenced by the Cancer Genome Atlas (TCGA). This cohort (the MCPyV-associated MCCs) 1) harbors clonal levels of MCPyV DNA, 2) expresses MCPyV T antigens, 3) generates antibodies to T antigens in their hosts, and 4) is found in areas characteristic of MCPyV-positive MCCs.\textsuperscript{19–21}

In contrast, the other cohort (the MCPyV-negative MCCs) is associated with an exceptionally high mutation burden (~40 mutations/Mb) that is higher than any cancer sequenced by the TCGA and ~100-fold higher than virus-positive MCCs (Figure 1a).\textsuperscript{19–21} These cancers do not show evidence of viral integration or expression of viral proteins. Additionally, they are preferentially found in anatomical locations characteristic of MCPyV-negative MCCs like the head and neck.\textsuperscript{26}

In theory, differences in mutation burden may be due to differences in genetic drivers. For example, virally-driven cancers may harbor fewer mutations than non-virally-driven cancers, because the expression of viral oncogenes obviates the need for additional cancer-promoting mutations. While this may be true, a comparison to head and neck squamous cell carcinomas (HNSCCs) suggest that other factors may be at play (Figure 1a). Like MCCs, HNSCCs are either virus-independent or associated with a virus that encodes viral oncogenes (human papillomavirus). In contrast to MCCs, virus-associated and virus-negative HNSCCs have little to no differences in the mutational burden.\textsuperscript{27, 28}

The types of mutations are also profoundly different between MCPyV-negative and MCPyV-positive MCCs. The mutational signature of MCPyV-positive MCC does not show evidence of any exposure to known mutagens. Instead, the MCPyV-positive mutational signature is typical of mutations that accumulate during aging. In contrast, MCPyV-negative MCCs harbor a strong ultraviolet signature.\textsuperscript{19, 21, 29} These data strongly suggest that the cell of origin of the MCPyV-negative MCC but not of the MCPyV-positive MCC has been extensively exposed to ultraviolet light. As mentioned above, this genetic difference is supported by the epidemiological data showing relative dominance of the MCPyV-negative tumors in Australia.\textsuperscript{15}

One potential explanation is that MCPyV-positive MCCs occur strictly in sun-shielded areas. However, we know from epidemiological data that, with a few exceptions, this is not the
We therefore posit an alternative explanation: the cell of origin for MCC originates in two distinct niches in the skin, with one protected from UV light (for MCPyV-positive MCC), and the other residing in a compartment with heavy exposure to UV irradiation (for MCPyV-negative MCC).

**Merkel cells may not be the cell of origin for MCCs**

Even though MCCs share certain features with Merkel cells, emerging data suggest that Merkel cells are not the cells of origin. Merkel cells are oval shaped osmoreceptors and mechanoreceptors essential for light touch sensation. They reside in the stratum basale. This causes our first conflict - while Merkel cells are fundamentally epidermal, the vast majority of MCCs are dermal or subcuticular. Second, while MCCs share some markers with Merkel cells, there are important differences. For example, although cytokeratin 20 (CK20) is expressed in both Merkel cells and MCCs, its pattern of expression differs sharply. In Merkel cells, CK20 is loosely arranged, leading to diffuse CK20 immunohistochemical staining in the cytoplasm. In contrast, in MCCs, CK20 are organized in characteristic whorl- or plaque-like arrangements, leading to the classic dot-like staining pattern on IHC. Third, MCCs express a number of markers such as c-kit, CD171, and CD24 that are not expressed in Merkel cells. Fourth, Merkel cells appear to be terminally differentiated with limited proliferative potential and slow turnover. Dying Merkel cells appear to be replaced not by proliferating Merkel cells but rather by differentiated pluripotent epidermal stem cells. These data may explain the absence of data supporting proliferation of Merkel cells in vivo. For instance, many other cells from the skin proliferate leading to the appearance of benign tumors, e.g. fibroblasts, fibromas; sebocytes, sebaceous adenomas; adipocytes, lipomas; melanocytes, nevi; keratinocytes, seborrheic keratosis. In contrast, there are no known benign Merkel cell tumors.

**Failure to generate Mouse Models of MCC using Merkel cell-specific Cre drivers**

Efforts to generate mouse models of MCC utilizing Merkel cell-specific Cre drivers have largely failed. MCPyV-negative MCCs have frequent mutations in TP53 (73%–100%) and RB1 (45%–100%), and less commonly but notably in the PI-3-kinase pathway (up to 18% of cases). To develop an in vivo model of MCPyV-negative MCCs, the Sage lab generated mutant mice in a C57BL/6;129Sv/J mixed genetic background with deletions of relevant tumor suppressors (Tp53, three components of the RB family (Rb1, p107, and p130); and a component of the PI-3-kinase pathway (Pten)). Triple knockout (TKO) mice harbored floxed alleles in Tp53, Rb1, and p130 (as described in). Quadruple knockout (QKO) mice also harbored floxed alleles in Pten or a null allele for p107. These mice were crossed with mice harboring Cre-ERT2 expressed from the Atoh1 promoter. Atoh1 encodes a lineage-specific protein (ATOH1, also known as MATH1) that is expressed in Merkel cells and is required for Merkel cell differentiation. In these mice, low frequencies of Merkel cells were found to re-enter the cell cycle 5 days after Tamoxifen administration (no more than 20% – N.J. and J.S., data not shown). Through the end of study (12 to 15 months after Tamoxifen administration), no advanced and aggressive tumors were formed (Table 1).
few benign lesions were observed on the ears or the back of mice (N.J. and J.S., data not shown). To rule out the role of additional UV-induced mutations, *Atoh1-CreERT2; TKO* and *Atoh1-CreERT2; QKO* mice were exposed to daily UV-B at 300 J/m² for two weeks. None of these mice developed MCCs. Strikingly, parallel experiments with similar mutant alleles in similar genetic backgrounds show efficient tumor initiation and development of aggressive neuroendocrine lung cancer. These negative results suggest that adult Merkel cells are not a likely cell of origin for MCPyV-negative MCC.

To make a mouse model of MCPyV-positive MCCs, Shuda et al. conditionally expressed the small T antigen from Merkel cells (Table 1). This model is reasonable because the vast majority of MCPyV-positive MCCs do not have cancer-promoting somatic mutations. In addition, although both LT and ST antigens appear to contribute to proliferation and survival 

**An epidermal origin of MCPyV-negative MCC**

The genomic data suggest that the epidermal keratinocyte may be the cell of origin for MCPyV-negative MCCs. First, MCPyV-negative MCCs harbor a UV mutational signature, which is characteristic of epidermal-derived cancers. 86% of mutations in MCPyV-negative MCCs are C>T transitions, the majority of which (66% of total) have the trinucleotide context characteristic of UV-mediated DNA damage. The same UV signature is found only in proliferations of epidermal cells, including keratinocyte clones in “normal” eyelid skin, actinic keratosis, and squamous cell carcinoma (SCC), melanocytic nevi and melanomas, and even cutaneous T cell lymphomas (Figure 1a).

Secondly, mutation burden in epidermal cancers is a function of the specific epidermal cell of origin. Cutaneous T cell lymphomas harbor ~3.2 mutations/Mb, melanomas ~14 mutations/Mb, and keratinocyte derived basal cell or squamous cell carcinomas 50–75 mutations/Mb. With a median of ~40 mutations/Mb and over 1000 SNVs per exome, the mutational burden of MCPyV-negative MCCs is significantly higher than any cancer
sequenced by the TCGA including melanomas and is in line only with two other cancer types, both keratinocyte-derived skin cancers (Figure 1a). In addition to the overall mutational burden being compatible with a keratinocyte origin, MCPyV-negative MCC harbor mutations in NOTCH1, HRAS, and FAT1 that are also frequently mutated in SCCs. Other orthogonal data support this hypothesis. Many other skin cancers can be observed in collision tumors between the cancers themselves and precursor lesions derived from the same cell of origin. This is true for both melanomas, which may arise within melanocytic nevi, and SCCs, which can be found contiguous with actinic keratoses. There are no reported cases of collision tumors of MCCs and benign proliferations of Merkel cells, but there are 18 case reports of collision tumors between MCCs and keratinocytic neoplasms, such as SCC and SCC in situ. In all of these cases, the MCCs are MCPyV-negative, had an extremely high mutational burden, and had a direct physical connection to epidermal keratinocytes. Given that ~80% of MCCs are viral-associated, the absence of MCPyV-positive MCCs in these collision tumors is unlikely to occur by chance alone (p<0.0001; chi-square test). These findings instead suggest that these collision tumors occur because MCCs, like SCCs, are derived from keratinocytes. Alternatively, both SCCs and MCCs can arise from the same epidermal stem cell population, which can differentiate into both keratinocytes and Merkel cells (Figure 1b).

A dermal origin for MCPyV-positive MCC

In contrast, the genetic data suggest that the MCPyV-positive MCCs may be derived from a completely distinct, dermal cell of origin. Unlike any epidermal-derived cancers, MCPyV-positive MCCs harbor very few mutations. The few mutations that are found do not harbor a UV signature (Figure 1a). These data would appear to exclude epidermal cells, e.g. epidermal keratinocytes, melanocytes, and Merkel cells, as the cell of origin of MCPyV-positive MCCs. Such data highlight the potential for dramatic differences in the pathophysiology of MCPyV and MCPyV-positive MCCs.

Recent virology experiments suggest that dermal fibroblasts may be the target of MCPyV infection and thus a putative cell of origin for MCPyV-positive MCCs. Liu and colleagues infected the skin with both a MCPyV-Green Fluorescent Protein (GFP) pseudovirus and the full MCPyV virus. They found that while MCPyV can deliver reporter DNA to a wide variety of cells in the skin, MCPyV could not infect CK20-positive Merkel cells. Moreover, only dermal cells expressing fibroblast markers were capable of expressing the MCPyV LT and ST antigens. Lastly, they showed that ex vivo, dermal fibroblasts were the only cell types that can support the full viral life cycle: viral entry, viral transcription, and viral replication. Consistent with their potential role in MCC initiation, dermal fibroblasts have a mutation burden (0.15–0.37 mutations/Mb) and mutational signature similar to MCPyV-positive MCCs.

Neuroendocrine transdifferentiation via inhibition of RB

If the two main MCC subtypes are derived from different cells of origin, how could they look similar pathologically? Multiple lines of evidence support the possibility of cancer cell
plasticity, wherein divergent cell types can acquire neuroendocrine phenotypes. The molecular mechanism underlying this lineage plasticity remains unclear; however, many of these phenotypic changes appear to result as a consequence of dysregulation of RB and/or p53.  

Like MCPyV LT antigen, the LT antigen of a related polyomavirus (SV40) inhibits RB. Ectopic expression of SV40 LT antigen is sufficient to induce neuroendocrine transdifferentiation in mouse models of gastric and prostate cancer even when initially expressed from non-neuroendocrine cells. In a model of gastric cancer, SV40 LT antigens were expressed from gastric epithelial cells using a gastric parietal cell-specific Cre driver (Atp4b-Cre). Instead of developing gastric adenocarcinoma, as originally anticipated, these mice instead developed exclusively neuroendocrine carcinomas. Similarly, in a mouse model of prostate cancer, SV40 T antigens are expressed in prostate epithelial cells using probasin promoters as a driver. These mice initially develop adenocarcinoma followed by an epithelial to neuroendocrine transition during tumor progression and metastasis.

In humans, RB1 and TP53 mutations confer drug resistance in human prostate cancer and in EGFR mutant lung cancer. Remarkably, these mutations appear to induce epigenetic reprogramming of the cancer cells leading to lineage plasticity. This is characterized by induction of chromatin modifying enzymes, e.g. EZH2, and transcription factors, e.g. SOX2, leading to dramatic phenotypic changes, including appearance of neuroendocrine features. In lung cancer, EGFR mutant lung adenocarcinomas that are resistant to tyrosine kinase inhibitors can acquire a neuroendocrine small cell lung cancer phenotype. Because RB1 and TP53 are frequently mutated in MCPyV-negative MCCs, these mutations may promote transdifferentiation into a Merkel cell-like, neuroendocrine phenotype.

Conclusions

Despite considerable recent advances in MCC biology and treatment, the cell of origin of MCCs remains in question. Parsing out the true cells of origin may be important for the development of relevant pre-clinical models and an eventual cure. Recent genomics studies suggest that MCPyV-negative and -positive MCCs are distinct entities, arising from distinct cells-of-origin residing in different parts of the skin. Conceptually this leads us to propose that MCCs results from convergent tumor evolution, whereby two different cells of origin transdifferentiate into a shared neuroendocrine phenotype through inhibition of RB either through somatic mutations or expression of LT and ST antigens (Figure 1b).

This model may be testable utilizing a combination of epigenetic techniques and mouse models. Unbiased approaches such as ATAC-seq and RNA-seq may show that MCCs retain epigenetic features that are shared with their cell of origin, i.e. keratinocytes and dermal fibroblasts. Similarly, alternative keratinocyte-derived (or epidermal stem cell-derived) or dermal fibroblast Cre-drivers, respectively, may be able to functionally demonstrate the ability of these cells in vivo to initiate models of virus-negative and virus-positive MCCs, respectively. Development of such models could provide for a robust platform for preclinical development of MCC targeted therapeutics.
Acknowledgments

J.C. is the Ruth K. Freinkel Assistant Professor of Dermatology. J.C. is supported by the NCI (K08 CA 191019). J.S. is the Harriet and Mary Zelencik Scientist in Children’s Cancer and Blood Diseases. The mouse work was supported by the NCI (R21 CA167104-01). Mice were maintained according to practices prescribed by the NIH at Stanford’s Research Animal Facility accredited by the AAALAC (protocol 13565). We thank Dr. David MacPherson for the Phxmutant mice and Dr. Anton Berns for the Tp53mutant mice. We would like to thank everyone in the Sage laboratory who helped in the generation and characterization of all the mutant mouse cohorts, specifically Margaret Zhu, Kim Tran, Garrett Seitz, and Anuradha Talihreddy, as well as Dr. Jinah Kim for help with the histopathology.

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Figure 1.
(A) Mutational Burden of Skin and Selected Non-Skin Cancers for Comparison
Blue bars = dominant UV signature. Red bars = non-dominant/lack UV signature. BCC = Basal Cell Carcinoma; SCC = Cutaneous Squamous Cell Carcinoma; MCPyV− or + MCC = Merkel Cell Polyoma Virus negative or positive Merkel Cell Carcinoma; AK = Actinic Keratosis; Cutan-Mel = Cutaneous Melanoma; Lung SCC = Lung Adenocarcinoma; HNSCC = Head and Neck Squamous Cell Carcinoma; KC = Keratinocyte; CTCL = Cutaneous T cell Lymphoma; Acral Melanoma; Breast CA = Breast Adenocarcinoma

(B) Schema Depicting Model of Convergent Development of MCPyV− and MCPyV+ MCC.
In the epidermis, a keratinocyte precursor is depicted undergoing UV mutagenesis, positive selection, mutation in Rb and p53, and neuroendocrine transdifferentiation resulting in MCPyV− MCC. In the dermis, MCPyV enters dermal fibroblasts, undergoes LT truncation leading to viral repression of Rb, sustained ST expression, and eventual virus-induced neuroendocrine transdifferentiation resulting in MCPyV+ MCC.
Table 1

Mouse models of MCC

| Model                                                                 | Phenotype                                                                 | Reference                                      |
|-----------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------|
| Developmental induction of MCPyV early region (with small T and large T antigens) in K14-positive cells (K14-cre Lox-stop-Lox MCPyV early) | Mice develop hyperplasia, hyperkeratosis, and acanthosis of the skin, some mice develop cutaneous papillomas – some proliferation in Merkel cells | Spurgeon et al. Cancer Res, 2015\(^2\)         |
| Postnatal induction of MCPyV small T antigen in K5-positive cells (K5-creER Lox-stop-Lox ST Ag) | Epidermal transformation and squamous cell carcinoma in situ – No expression of MCC markers in skin lesions | Verhaegen et al. J Invest Dermatol, 2015\(^3\) |
| Postnatal induction of small T antigen and loss of p53 in Atoh1-positive cells (Atoh1-creER Lox-stop-Lox ST Ag and p53 flox/flox) | Some effects of ST expression in Atoh1-positive cells during embryonic development but no effects on Merkel cells numbers and no MCC in adults | Shuda et al. J Invest Dermatol, 2015\(^4\)     |
| Developmental induction of MCPyV small T and large T antigens and Atoh1 under the control of the K5 promoter in transgenic mice | MCC-like intraepidermal lesions in pre-term embryos with expression of Atoh1 and ST – No additional effect of LT | Verhaegen et al. Cancer Res, 2017\(^5\)       |
| Postnatal deletion of tumor suppressor genes in Atoh1-positive cells (Atoh1-creER, and combinations of p53 flox/flox, Rb flox/flox, p130 flox/flox, pten flox/flox, and p107 null) | Combined deletion of Rb/p53/p130, Rb/p53/p130/p107, or Rb/p53/pten after induction of Cre by tamoxifen in nursing mothers or young adult mice + groups of mice exposed to ultraviolet light – proliferation in ~20% of Merkel cells, small lesions observed mostly on ears in around 10–30% of mice, no MCC (over 20 mice tested per cohort, aged at least 12 months) | Jahchan and Sage unpublished                  |