The Origins of Merkel Cell Carcinoma: Defining Paths to the Neuroendocrine Phenotype

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Two recent reports leverage careful genomic analysis to show that human Merkel cell carcinomas (MCCs) arising from squamous cell carcinoma in situ bear hallmarks of genetic relatedness, suggesting that a subset of MCCs can arise from pre-existing intraepithelial keratinocytic proliferations in human skin. This has important implications for our understanding of cells of origin and the diverse drivers of MCC formation.

Introduction

Primary neuroendocrine carcinoma of the skin, eponymously termed Merkel cell carcinoma (MCC), is rare, with an estimated incidence of 0.2–0.7 per 100,000 annually in the United States and significant lethality (Harms et al., 2018). There are two established etiologies. The more common one is due to the integration of an altered Merkel cell polyomavirus (MCPyV) genome with the expression of viral oncoproteins and low tumor mutation burden (TMB). In the second, characteristic mutations in R81 and TP53 occur in the context of UV signature mutations (Goh et al., 2016; Knepper et al., 2019). Immunotherapy has proven very effective, yet there are no targeted agents and no definitive strategies for recurrent or immunotherapy-refractory disease.

The conundrum of how such widely divergent genomic profiles can give rise to what are largely indistinguishable tumors even from the perspective of therapeutic responses remains largely unanswered. Part and parcel of this is the identification of the cell of origin of MCC. The previously long-standing assumption that the cell of origin is normal Merkel cells is still a matter of intense scrutiny and debate. Candidate origins have included Merkel cells, fibroblasts, and B cells (Harms et al., 2018). Multiple reports (Pulitzer et al., 2015) point to relatively common occurrences of divergent histologies presented in combined squamous cell carcinoma (SCC)–MCC tumors, in which areas of squamoid differentiation are apparent. This is analogous to what occurs in prostate and bladder carcinomas, where neuroendocrine (trans) differentiation portends poor clinical outcomes. Sunshine et al. (2018) have commented specifically on how multiple cells of origin may exist, reasoning that the dichotomy in genomic profiles, the requirement for UV-mediated DNA mutagenesis in one subset, and the establishment of a productive in vitro viral replication cycle only in human fibroblasts are collectively quite consistent with this concept.

MCC can arise from SCC in situ

Two recent reports shed additional light on these important issues. Kervarrec et al. (2021) profiled four pairs of histologically contiguous SCC in situ (SCCIS) and MCC arising on sun-damaged skin by microdissecting histologically distinct areas and subjecting recovered tissue to whole-exome sequencing. Over 750 mutations per segregated tumor were identified; the MCC portion had a higher mutational load, and all were dominated by UV signature. All MCCs expressed classical markers such as CK20, chromogranin/synaptophysin, and CD56. Overlapping R81 mutations were identified in paired SCCIS and MCC components, and SOX2 was strongly expressed in both. These are both features that are not typically observed in sporadic cutaneous SCC, and SOX2 has been placed upstream of ATOH1, a key neuroendocrine transcription factor that is often upregulated in typical MCC. Finally, H3K4 monomethylation and H3K27 acetylation were significantly reduced in MCC compared with those in distinct samples of other skin carcinomas.

A complementary report by Harms et al. (2021) interrogated seven SCCIS–MCC pairs using targeted DNA sequencing and RNA sequencing performed on microdissected compartments separately. Again in this experiment, clear spatial segregation was noted histologically, and similar classical MCC-associated marker expression was noted, including that of cytokeratins 8 and 20. Genetic relatedness was demonstrated through the identification of common TP53 and R81 mutations observed in six of seven SCCIS–MCC pairs. In addition, shared copy number gains in MYCL and MDM4 were observed, as were shared mutations in NOTCH2, KMT2C, and KMT2D, which have all been identified in typical UV-induced MCC.

The transcriptomes of the SCCIS-associated MCC portions were enhanced for neuronal signatures and polycomb repressive complex (PRC) targets as well as NCAM1, keratin 20 gene K20, and PIEZ02. Consonant with this signal from PRC target upregulation, H3K27 trimethylation was consistently decreased relative to that of the adjacent SCCIS component, as was HLA-A expression, a class I major histocompatibility complex allele often epigenetically silenced in MCC.

Conclusions

Taken together, these important findings show that at least within this relatively
Clinical Implications

- Merkel cell carcinomas (MCCs) can arise in association with invasive and in situ squamous cell carcinomas (SCCs).
- Genomic analyses of combined SCC in situ (SCCIS)—MCC lesions show common alterations in multiple genes, including key tumor suppressors RB1 and TP53 present in both areas.
- SCCIS-associated MCC are enriched for polycomb repressor complex targets, implicating an epigenetic switch to neuroendocrine differentiation.

Small cohort of combined SCCIS/MCC lesions, several important conclusions can be drawn (Figure 1). First, on the basis of the mutational profiles and greater number of mutations in MCC than in SCCIS, MCPyV-negative MCC can arise from SCCIS. Second, although no mutational event clearly appears to be required for the MCC transition, RB1 inactivation is likely obligate, especially given the additional decrease in expression in the MCC component noted by Harms et al. (2021). Clearly, RB1 is a vital target by virtue of its common inactivation in neuroendocrine carcinomas as well as being the cellular target of MCPyV large T antigen. Finally, the clear upregulation of PRC-associated target genes and decreased H3K27 trimethylation relative to that of the adjacent SCCIS implicate epigenetic mechanisms in the creation and maintenance of the MCC neuroendocrine phenotype.

Recently, LSD1 activity has been established as essential for the viability of MCPyV-driven MCC, and inhibition of LSD1 activity selectively drives differentiation and cell death. Data from RNA interference–based and CRISPR-based screens implicate a group of additional epigenetic regulators, including BRD4, PRMT5, and noncanonical BAF signaling (Leiendecker et al., 2020; Park et al., 2020). In comparing the SCCIS component with the adjacent MCC component, Harms et al. (2021) show lower H3K27me3 levels in the MCC component. In comparing the MCC components with a distinct set of skin carcinomas, Kervarrec et al. (2021) found lower H3K27me1 and H3K27ac levels. These may seem to be contradictory because H3K27me3 is associated with closed chromatin and transcriptional repression, whereas H3K27ac is associated with open chromatin. However, the comparisons made were not identical, and Harms et al. (2021) rightly posit that MCC-associated SCCIS components represent a minority subset of RB1-inactivated SCCIS. They are thus unusually permissive for a neuroendocrine switch, occupying an intermediate unique genetic and epigenetic state between typical cutaneous SCCIS or SCC and UV-induced MCC.

Collectively, these findings suggest that multiple epigenetic mechanisms are required for MCC to simultaneously maintain their distinctive neuroendocrine differentiation while enabling tumorigenic features. A further understanding of how this is accomplished may be gained by placing this in the context of other neuroendocrine carcinomas. A key clinicopathologic differential diagnosis to be excluded in the diagnosis of MCC is small cell lung carcinoma (SCLC), which bears hallmarks of a high TMB cancer with a predominant tobacco exposure signature. Recently, several reports detail a spectrum of neuroendocrine differentiation that is associated with the expression of transcription factors NEUROD1, ASCL1, and POU2F3 and a subgroup with high YAP/TAZ activity and low neuroendocrine differentiation (Rudin et al., 2019).

Finally, the genomic contrast between UV-induced and MCPyV-induced MCC suggests that MCPyV mutation, integration, and oncoprotein expression are sufficient to generate tumors. This, in turn, likely reflects a much more restricted path to oncogenesis through MCPyV integration and, by extension, suggests a more consistent panel of molecular alterations and vulnerabilities. In contrast, although there are still near-universal molecular events such as inactivation of TP53 and RB1 in the context of UV-induced high TMB MCC, there is likely a significantly wider diversity of molecular paths to generating MCC akin to what is observed in SCLC. It remains to be seen whether molecularly defined subsets of UV-induced MCC may be further deduced and which populations

**Figure 1. Summary of key findings in combined SCCIS–MCC tumors.** Major findings from the two highlighted reports are superimposed on a photomicrograph of a combined SCC (left)—MCC (right) tumor at the interface showing distinct histological features of typical SCC with keratinizing epithelial cells with eosinophilic cytoplasm, contrasted with features of MCC with small basophilic cells with scant cytoplasm and indistinct and stippled chromatin. Genomic and immunohistochemical analyses of combined SCCIS–MCC tumors show high TMB, with increased mutational burden in MCC. Classic MCC-associated marker expression was found in the MCC portion at the immunohistochemical and transcriptional levels. Many genomic alterations, including mutations in RB1 and TP53, were found in common between the two areas, in addition to copy number gains in MYCL and MDM4. In the MCC portions, PRC targets were upregulated in transcriptomic analysis, coinciding with upregulation of neuronal signatures and decreased H3K27me3. Bar = 50 μm. MCC, Merkel cell carcinoma; PRC, polycomb repressor complex; SCC, squamous cell carcinoma; SCCIS, squamous cell carcinoma in situ; TMB, tumor mutation burden.
of cells within the skin are capable of giving rise to the tumors now collectively called MCC. These two important reports show that one path is through a keratinocytic intraepidermal precursor and suggest that keratinocytes can serve as a cell of origin for MCPyV-negative MCC.

REFERENCES


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New Key Players in Irritant Contact Dermatitis: Residential Skin Cells and Neutrophils Drive Inflammation

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The chemokine CCL2 is a potential biomarker for progression of inflammatory skin disease. In a new article of the Journal of Investigative Dermatology, Shibuya et al. (2021) use murine experimental models to show that CCL2–CCR2–dependent IL-1β secretion by local skin cells and skin-infiltrating neutrophils are key drivers of skin irritation.

Irritant contact dermatitis is a common disease that lacks effective treatment. Irritant contact dermatitis (ICD) is an inflammatory skin disease that is caused by irritants, including chemicals, direct friction, and wet work. It is commonly expressed as hand eczema, and as such, it is an increasingly important cause of work-related disease (Reinholz et al., 2021). The mechanism of ICD is not well-characterized, and this hampers both differentiation from allergic contact dermatitis and initiation of proper treatment. ICD can be treated with topical and systemic corticosteroids, but responses to other therapies such as calcineurin inhibitors and UVB are less effective. In chronic cases, ICD can be treated with systemic retinoid alitretinoin (Diepgen et al., 2015). However, new treatments for this debilitating disease are greatly needed (Bonnekoh et al., 2021).

CCR2–CCL2 signaling drives skin inflammation

In a new article of the Journal of Investigative Dermatology, Shibuya et al. (2021) identify novel cellular mechanisms that are crucial for the induction of skin irritation through a complementary series of murine knock-out, chimeric, and parabiosis experiments. The authors hypothesized that the CCL2–CCR2 axis might be important in driving ICD-associated skin inflammation. CCL2 has previously been implicated as a key mediator of inflammation in various organs (Chen et al., 2020; Dimitrijevic et al., 2007; Wolf et al., 2019) as well as in inflammatory disorders, including atherosclerosis, rheumatoid arthritis, allergic