EDITORIAL

Evidence of an epithelial origin of Merkel cell carcinoma

Modern Pathology (2022) 35:446–448; https://doi.org/10.1038/s41379-021-00964-x

The current system of tumor classification relies largely on the recognition of a specific tumor cell morphology in conjunction with a unique immunophenotype[1]. Notably, molecular-genetic alterations occurring prior to or during tumor development contribute to the tumor cell development and its subsequent unique phenotype[2]. However, whether morphologic and genetic features always reflect the precise cell of origin remains controversial[2,3], and for some tumor types, a precise identification of the cell of origin is not always available[4]. However, this remains a critically important challenge, as such findings have important implications for diagnosis, therapy and prevention[3,5].

One such paradigm for this question occurs in Merkel cell carcinoma (MCC)[6–9]. MCC is a rare and aggressive high grade cutaneous neuroendocrine carcinoma occurring in sun exposed skin of elderly and/or immunosuppressed patients[10–12]. In 2008, genomic integration of the Merkel cell Polyomavirus (MCPyV) was identified as the primary oncogenic driver for about 80% of MCCs[13], while the remaining MCPyV-negative cases were subsequently shown to harbor a high tumor mutation burden with prominent UV-signature[14–18]. Although the physiological Merkel cell was initially considered as the MCC cell of origin due to close phenotypic similarities[12], this hypothesis has been dismissed as unlikely because mature Merkel cells are post-mitotic[19,20]. As such, they cannot be effectively infected by MCPyV[9,21], which requires entry into the cell cycle for successful integration and transformation. In keeping with this concept, ectopic overexpression of the MCPyV viral oncoproteins in transgenic mice failed to induce cell cycle reentry in mature Merkel cells[22].

Thus, several other candidate cells of origin for MCC have been proffered, including epithelial[6,9,23] and non-epithelial progenitors[7,8,24].

In this regard, epithelial stem cells represent the most likely progenitors of differentiated Merkel cells[20,25,26] and have been suggested as relevant candidates for the MCC cell of origin[6,9]. As for non-epithelial cells: since MCC frequently lack epidermal connection, demonstrate expression of B cell markers (notably TdT and PAX5) and in some cases have been reported to harbor immunoglobulin rearrangement, it has been suggested that MCPyV-positive MCC could derive from pre-/pro-B cells[7]. Another hypothesis is that MCPyV-positive cases derive from dermal mesenchymal cells, based on the identification of dermal fibroblasts as productive hosts of MCPyV infection[21], as well as their deep location and the low tumor mutational burden together with a lack of UV signature[8]. Finally, a recent study demonstrating that MCPyV oncoprotein knock down in MCC cell lines leads to the acquisition of a differentiated neuronal phenotype in certain contexts might indicate that MCC could actually derive from a neuronal lineage[24].

Importantly, all of these theories imply that phenotypic changes with acquisition of neuroendocrine and "Merkel cell-like" features arise during tumor cell development[6]. Notably, in small cell lung cancer, a neoplasm that shares close phenotypic similarities with MCC, dual inactivation of *TP53* and *RB1* are regarded as the principle oncogenic drivers inducing both transformation and neuroendocrine differentiation in epithelial cells[27,28]. Similarly, in MCC, expression of the RB1-inactivating MCPyV oncoprotein Large T antigen[29] induces transcription and/or protein accumulation of *Atonal homolog 1 (ATOH1)*[30,31] and *SRY-box 2 (SOX2)*[24], two transcription factors critically involved in Merkel cell differentiation[20,25,26]. Although these findings might suggest that expression of the MCPyV oncoproteins in a skin epithelial progenitor results per se in cell transformation and acquisition of a Merkel cell-like phenotype, several transgenic mice models demonstrated that MCPyV oncoprotein expression alone was not sufficient to mediate this phenotypic switch[22,32–34]. Furthermore, viral oncoprotein expression does not explain the origin of MCPyV-negative MCCs.

In this issue of *Modern Pathology*, Harms *et al*. collected a series of rare tumors to address the question of the origin of virus-negative MCC in a systematic manner. A subset of MCCs arise in association with squamous cell carcinoma in situ (SCCIS), which provides the opportunity to query the molecular genetic alterations in each component to determine a possible etiologic relationship between the two. Harms *et al*., applied targeted next generation sequencing to seven paired in situ squamous cell carcinoma (SCCIS)-MCPyV-negative MCC samples, sequencing these components separately. Their results strongly suggest a direct clonal association by demonstrating high mutational similarities between the two tumor components. Notably in almost all cases, both SCCIS and MCC harbored common *TP53* and *RB1* mutations and/or deletion, while other alterations previously reported as less common in MCPyV-negative MCC, notably *MYC-L* and *MDM4* amplifications[35,36], were only present in a minority of cases. Interestingly, although they observed *FBXW7* copy loss or mutation and *SMARCA4* mutations restricted to the MCC component in several cases, they could not identify a universal driver mutation for the squamous to neuroendocrine transition. Transcriptomic analysis of 4 paired cases in comparison to pure SCCIS with ($n = 4$) and without ($n = 5$) *RB1* mutations further revealed that SCCIS associated with MCC formed a cluster distinct from other SCCIS and adjacent to *RB1*-inactivated SCCIS. This analysis additionally demonstrated enrichment of the neuronal and Merkel cell markers together with upregulation of targets of the Polycomb Repressive Complex, and repression of the immune and inflammatory genes, upon SCCIS to MCC transition. Finally, applying digital quantitation of immunohistochemical protein expression, the authors confirmed induction of SOX2, together with downregulation of RB1, H3K27 trimethylation, and HLA-A during the SCCIS to MCC transition. The results presented by Harms *et al*. strongly suggest that MCPyV-negative MCC can arise from a distinctive subset of SCCIS harboring specific genetic alterations such as *TP53* and *RB1* inactivation. Epigenetic dysregulations then probably further contribute to the SCC to MCC transformation.

Received: 21 September 2021 Revised: 18 October 2021 Accepted: 18 October 2021
Published online: 3 November 2021

These results were independently confirmed in parallel observations from our own group. Indeed, we recently demonstrated a clonal link between SCC and MCC by applying whole exome sequencing to four combined MCC cases[37]. To this end, we identified a large number of somatic variants present in both the SCC and MCC components ($n = 69–1060$), with allelic frequencies higher in the MCC components suggesting that the latter may have derived from an SCC cell. And in complete agreement with the Harms et al. study, the comparison of the SCC and MCC parts did not allow us to identify a specific oncogenic driver contributing to the neuroendocrine transition. By contrast, immunohistochemical analysis revealed reduced histone H3K4 methylation and H3K27 acetylation in the MCC component, suggesting that epigenetic changes likely contribute to the transformation. Finally, by comparing mutation frequencies observed in our data set to those detected in previously published MCPyV-negative MCC ($n = 43$) and cutaneous SCC ($n = 98$) cases, we demonstrated that RB1 inactivation constitutes an early mandatory but obviously not sufficient step for the transformation of SCC to MCPyV-negative MCC. In mirroring each other's results, these paired publications represent a critical achievement in our understanding of MCC biology[5] by clearly demonstrating an epidermal origin of some MCPyV-negative cases.

However, it is unlikely that these findings can necessarily be generalized to all MCC cases including MCPyV-positive and MCPyV-negative tumors. A tumor cell's phenotype results from the changes induced within the cell of origin by the constellation of molecular-genetic alterations (somatic mutations, amplifications/deletions, fusions and/or epigenetic alterations) during tumor development and progression[2]. As an example, mutations affecting beta-catenin (*CTNNB1*) together with mutations that result in MAPK pathway activation produce a melanocytic nevus with deep penetrating nevus morphology[38], while the same genetic alteration in the skin epithelial lineage is observed in tumors with matrical differentiation[39,40]. Although not a formal proof, these findings strongly suggest that expression of the same oncogenic driver in distinct cell lineages results in the development of distinct tumor types[2]. Notably, although at least three transcriptional pathways are generally deregulated in MCC either by MCPyV or by mutations in virus negative MCC[41], there are significant variations in morphology, immunophenotype, genetic backgrounds and clinical outcome observed between MCPyV-positive and MCPyV-negative cases[15,42–44]. Hence, it is possible that MCPyV-positive and MCPyV-negative cases might actually represent distinct tumor entities[14,42,45–47] with potentially different cells of origin[8]. With regard to this hypothesis, we recently applied next generation sequencing to demonstrate MCPyV integration in trichoblastoma, a benign adnexal tumor harboring hair follicle differentiation, could give rise to an MCPyV-positive MCC[23]. This observation suggests that some MCPyV-positive cases may also derive from an epithelial lineage and further raises the possibility that hair follicle cells might constitute a potential ancestry of MCPyV-positive cases. In line with this hypothesis, ectopic expression of MCPyV proteins in epidermal and hair follicle cells resulted in expression of some Merkel cell markers[31,48]. Moreover, although some characteristic features of MCPyV-positive MCC such as deep dermal location, lack of intraepidermal involvement in most cases, and low mutational burden, and absence of UV mutation signature, have been used to support a non-epithelial origin, these findings might also be explained by a hair follicle origin[7,8,6]. In contrast, intraepidermal involvement, high tumor burden and presence of UV signature in MCPyV-negative cases are in accordance with an epidermal (interfollicular) origin as demonstrated by our findings and the current study.

Important questions remain regarding the origins of MCC. Although there is compelling evidence for an epithelial origin (regardless of viral status), what biological events explain the apparent lack of direct physical association between many MCC tumors and the adjacent epidermis or hair follicle from which they derive? Can a precise molecular switch for neuroendocrine differentiation in cutaneous epithelial precursors be defined—and conversely, what might revert MCC cells to a squamous phenotype? Finally, how can these events be recapitulated in transgenic mice to generate better models of MCC tumorigenesis? A great deal of exciting work remains, but we predict that these observations on the transition from SCC to MCC likely hold critical clues for answering such questions and unlocking additional mysteries about the origins of this aggressive neuroendocrine carcinoma.

Kervarrec Thibault [1,2]✉
[1]*Department of Pathology, University Hospital Center of Tours, Tours, France.* [2]*"Biologie des infections à Polyomavirus" team, UMR INRA ISP1282, University of Tours, Tours, France.*
✉*email: thibaultkervarrec@yahoo.fr*

REFERENCES

1. WHO. WHO classification of skin tumours, 4th edition. Lyon: International Agency for Research on Cancer, 2018.
2. Visvader, J. E. Cells of origin in cancer. *Nature* **469**, 314–322 (2011).
3. Becker, J. C. & Zur Hausen, A. Cells of origin in skin cancer. *J. Invest. Dermatol.* **134**, 2491–2493 (2014).
4. Blanpain, C. Tracing the cellular origin of cancer. *Nat. Cell Biol.* **15**, 126–134 (2014).
5. Harms, P. W. et al. The biology and treatment of Merkel cell carcinoma: current understanding and research priorities. *Nat. Rev. Clin. Oncol.* **15**, 763–776 (2018).
6. Kervarrec, T. et al. Histogenesis of Merkel cell carcinoma: a comprehensive review. *Front. Oncology.* **9**, 451 (2019).
7. Zur Hausen, A., Rennspiess, D., Winnepenninckx, V., Speel, E. J. & Kurz, A. K. Early B-cell differentiation in Merkel cell carcinomas: clues to cellular ancestry. *Cancer Res.* **73**, 4982–4987 (2018).
8. Sunshine, J. C., Jahchan, N. S., Sage, J. & Choi, J. Are there multiple cells of origin of Merkel cell carcinoma? *Oncogene* **37**, 1409–1416 (2018).
9. Tilling, T. & Moll, I. Which are the cells of origin in merkel cell carcinoma? *J. Skin Cancer*. **680410**, 36–42 (2012).
10. Schadendorf, D. et al. Merkel cell carcinoma: epidemiology, prognosis, therapy and unmet medical needs. *Eur. J. Cancer* **71**, 53–69 (2017).
11. Tetzlaff, M. T. & Harms, P. W. Danger is only skin deep: aggressive epidermal carcinomas. An overview of the diagnosis, demographics, molecular-genetics, staging, prognostic biomarkers, and therapeutic advances in Merkel cell carcinoma. *Mod. Pathol.* **33**, 42–55 (2020).
12. Tang, C. K. & Toker, C. Trabecular carcinoma of the skin: an ultrastructural study. *Cancer* **42**, 2311–2321 (1978).
13. Feng, H., Shuda, M., Chang, Y. & Moore, P. S. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* **319**, 1096–1100 (2008).
14. Carter, M. D. et al. Genetic profiles of different subsets of Merkel cell carcinoma show links between combined and pure MCPyV-negative tumors. *Hum. Pathol.* **71**, 117–125 (2018).
15. Harms, P. W. et al. The Distinctive Mutational Spectra of Polyomavirus-Negative Merkel Cell Carcinoma. *Cancer Res.* **75**, 3720–3727 (2015).
16. Harms, P. W. et al. Next generation sequencing of Cytokeratin 20-negative Merkel cell carcinoma reveals ultraviolet-signature mutations and recurrent TP53 and RB1 inactivation. *Mod. Pathol.* **29**, 240–248 (2016).
17. Wong, S. Q. et al. UV-associated mutations underlie the etiology of MCV-negative merkel cell carcinomas. *Cancer Res.* **75**, 5228–5234 (2016).
18. Goh, S., Lindau, C., Tiveljung-Lindell, A. & Allander, T. Merkel cell polyomavirus in respiratory tract secretions. *Emerg. Infect. Dis.* **15**, 489–491 (2009).
19. Moll, I., Zieger, W. & Schmelz, M. Proliferative Merkel cells were not detected in human skin. *Arch Dermatol Res* **288**, 184–187 (1996).
20. Ostrowski, S. M., Wright, M. C., Bolock, A. M., Geng, X. & Maricich, S. M. Ectopic Atoh1 expression drives Merkel cell production in embryonic, postnatal and adult mouse epidermis. *Development* **142**, 2533–2544 (2015).
21. Liu, W. et al. Identifying the target cells and mechanisms of merkel cell polyomavirus infection. *Cell Host Microbe* **19**, 775–787 (2016).
22. Shuda, M. et al. Merkel cell polyomavirus small T antigen induces cancer and embryonic Merkel cell proliferation in a transgenic mouse model. *PLoS ONE* **6**, 10 (2015).
23. Kervarrec, T. et al. Polyomavirus-positive merkel cell carcinoma derived from a trichoblastoma suggests an epithelial origin of this merkel cell carcinoma. *J Invest Dermatol* **140**, 976–985 (2020).

24. Harold, A. et al. Conversion of Sox2-dependent Merkel cell carcinoma to a differentiated neuron-like phenotype by T antigen inhibition. *Proc. Natl. Acad. Sci. USA.* **116**, 20104–20114 (2019).
25. Morrison, K. M., Miesegaes, G. R., Lumpkin, E. A. & Maricich, S. M. Mammalian Merkel cells are descended from the epidermal lineage. *Dev. Biol.* **336**, 76–83 (2009).
26. Perdigoto, C. N., Bardot, E. S., Valdes, V. J., Santoriello, F. J. & Ezhkova, E. Embryonic maturation of epidermal Merkel cells is controlled by a redundant transcription factor network. *Development* **141**, 4690–4696 (2014).
27. Park, J. W. et al. Reprogramming normal human epithelial tissues to a common, lethal neuroendocrine cancer lineage. *Science* **362**, 91–95 (2018).
28. Park, K.-S. et al. Characterization of the cell of origin for small cell lung cancer. *Cell Cycle* **10**, 2806–2815 (2011).
29. Houben, R. et al. An intact retinoblastoma protein-binding site in Merkel cell polyomavirus large T antigen is required for promoting growth of Merkel cell carcinoma cells. *Int. J. Cancer* **130**, 847–856 (2012).
30. Fan, K. et al. MCPyV large T antigen induced atonal homolog 1 (ATOH1) is a lineage-dependency oncogene in Merkel cell carcinoma. *J. Invest. Dermatol.* **140**, 56–65 (2019).
31. Kervarrec, T. et al. Merkel cell polyomavirus T antigens induce Merkel cell-like differentiation in GLI1-expressing epithelial cells. *Cancers* **21**, 12 (2020).
32. Spurgeon, M. E., Cheng, J., Bronson, R. T., Lambert, P. F. & DeCaprio, J. A. Tumorigenic activity of merkel cell polyomavirus T antigens expressed in the stratified epithelium of mice. *Cancer Res.* **75**, 1068–1079 (2015).
33. Verhaegen, M. E. et al. Merkel cell polyomavirus small T antigen initiates Merkel cell carcinoma-like tumor development in mice. *Cancer Res.* **77**, 3151–3157 (2017).
34. Verhaegen, M. E. et al. Merkel cell polyomavirus small T antigen is oncogenic in transgenic mice. *J Invest. Dermatol.* **135**, 1415–1424 (2015).
35. Paulson, K. G. et al. Array-CGH reveals recurrent genomic changes in Merkel cell carcinoma including amplification of L-Myc. *J. Invest. Dermatol.* **129**, 1547–1555 (2009).
36. Hill, N. T. et al. Distinct signatures of genomic copy number variants define subgroups of Merkel cell carcinoma tumors. *Cancers* **13**, 1134 (2021).
37. Kervarrec, T. et al. Merkel cell polyomavirus-negative -Merkel cell carcinoma originating from in situ squamous cell carcinoma: a keratinocytic tumor with neuroendocrine differentiation. *J. Investig. Dermatol.* S0022202X21021655 (2021).
38. Yeh, I. et al. Combined activation of MAP kinase pathway and β-catenin signaling cause deep penetrating nevi. *Nat Commun* **8**, 644 (2017).
39. Kyrpychova, L. et al. Basal cell carcinoma with matrical differentiation: clinicopathologic, immunohistochemical, and molecular biological study of 22 cases. *Am. J. Surg. Pathol.* **41**, 738–749 (2017).
40. Kazakov, D. V. et al. Mutations in exon 3 of the CTNNB1 gene (beta-catenin gene) in cutaneous adnexal tumors. *Am. J. Dermatopathol.* **31**, 248–255 (2009).
41. DeCaprio, J. A. Molecular pathogenesis of merkel cell carcinoma. *Annu. Rev. Pathol.* **16**, 69–91 (2021).
42. Harms, P. W. et al. Distinct gene expression profiles of viral- and nonviral-associated merkel cell carcinoma revealed by transcriptome analysis. *J. Invest. Dermatol.* **133**, 936–945 (2013).
43. Harms, K. L. et al. Virus-positive Merkel cell carcinoma is an independent prognostic group with distinct predictive biomarkers. *Clin. Cancer Res.* **27**, 2494–2504 (2021).
44. Moshiri, A. S. et al. Polyomavirus-negative Merkel cell carcinoma: a more aggressive subtype based on analysis of 282 cases using multimodal tumor virus detection. *J. Invest. Dermatol.* **137**, 819–827 (2017).
45. Starrett, G. J. et al. Clinical and molecular characterization of virus-positive and virus-negative Merkel cell carcinoma. *Genome Med.* **12**, 30 (2020).
46. Pasternak, S., Carter, M. D., Ly, T. Y., Doucette, S. & Walsh, N. M. Immunohistochemical profiles of different subsets of Merkel cell carcinoma. *Hum. Pathol* **82**, 232–238 (2018).
47. Kervarrec, T. et al. Morphologic and immunophenotypical features distinguishing Merkel cell polyomavirus-positive and -negative Merkel cell carcinoma. *Mod Pathol* **32**, 1605–1616 (2019).
48. Kervarrec, T., Chéret, J., Paus, R., Houben, R. & Schrama, D. Transduction-induced overexpression of Merkel cell T antigens in human hair follicles induces formation of pathological cell clusters with Merkel cell carcinoma-like phenotype. *Exp. Dermatol.* https://doi.org/10.1111/exd.14447 (2021).

AUTHOR CONTRIBUTIONS

TK performed writing, review and revision of the paper.

COMPETING INTERESTS

The author declares no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Kervarrec Thibault.