

# Targeting the epigenetic addiction of Merkel cell carcinoma

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**Merkel cell carcinoma (MCC) is a rare but very aggressive neuroendocrine cancer of the skin, with very limited therapeutic options. Although immunotherapy is effective in some cases, there is an unmet need for new therapeutic approaches in MCCs. In this issue of *EMBO Molecular Medicine*, Leiendecker *et al* identify a selective vulnerability of MCC for inhibitors of the lysine-specific histone demethylase 1A (LSD1). LSD1 inhibitors promote differentiation of tumor cells toward normal Merkel cell fate, impairing tumor cell growth *in vivo*, and opening new avenues for the treatment of patients with MCC.**

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See also: L Leiendecker *et al* (November 2020)

**M**erkel cells are neuroendocrine cells of the skin that are important for fine touch sensation (Lumpkin *et al*, 2010), arising from epidermal progenitors during embryonic development (Van Keymeulen *et al*, 2009). Their specification from epidermal cells relies on the activity of distinct pro-neural transcription factors including *Atoh1* and *Sox2* (Maricich *et al*, 2009; Van Keymeulen *et al*, 2009; Bardot *et al*, 2013).

Merkel cell carcinoma (MCC), which is thought to arise from Merkel cells, is a relatively rare but very aggressive neuroendocrine cancer of the skin, associated with advanced age and immunosuppression. Although it represents less than 1% of all non-melanoma skin cancers, its incidence has raised in the last years. Due to its very rapid metastatic spread, the mortality of

MCC is very high (33–46% mortality rate) (Harms *et al*, 2018). In 80% of the cases, it is caused by the integration of the Merkel cell polyomavirus (MCV), which promotes tumorigenesis through the cooperation of large T viral antigen, that inactivates the Retinoblastoma tumor suppressor gene, and small T viral antigen that activates the MYCL-EP400 transcriptional complex (Harms *et al*, 2018). Although immunotherapy is effective in a fraction of cases, the therapeutic options for the treatment of MCC are very limited (Nghiem *et al*, 2016).

Two recent studies, including one published in this issue of *EMBO Molecular Medicine* and the other recently published in *Nature Cell Biology*, identified LSD1 inhibition as a new therapeutic strategy to treat MCC (Leiendecker *et al*, 2020; Park *et al*, 2020). Using an *in vitro* drug screen with compounds targeting epigenetic regulators, Leiendecker *et al* identified an LSD1 inhibitor (LSD1i) as a specific and potent drug impairing the growth of MCV-positive MCC cell lines *in vitro*, without affecting the growth of skin fibroblasts. LSD1 is a histone demethylase that mediates the removal of mono- and dimethylation marks on H3K4, which are linked to active transcription, thus promoting repression of gene expression (Shi *et al*, 2004). ShRNA knockdown of LSD1 impairs the growth of MCC cells *in vitro*, similarly to LSD1 pharmacological inhibition.

To assess whether LSD1i was effective *in vivo*, the authors transplanted human MCC cell lines into immunodeficient mice and treated them with LSD1i. The treatment prevented tumor growth, whereas all control mice developed deadly tumors within 2 months. LSD1i also impaired the growth

of established tumors. The treated mice did not develop severe side effects, demonstrating the efficacy and tolerability of LSD1i for MCC treatment *in vivo*.

To assess the cellular mechanism by which LSD1i impairs MCC growth, the authors assessed MCC proliferation and cell death following treatment. LSD1i impaired MCC proliferation *in vitro* and *in vivo*, and induced MCC cell death by a caspase 3-dependent and caspase 3-independent mechanism.

To define the molecular mechanisms involved, Leiendecker *et al* performed a transcriptional profiling of MCC cells treated with LSD1i, and identified 870 upregulated and 533 downregulated genes compared to vehicle-treated cells. The downregulated genes include many genes regulating cell cycle and DNA replication, whereas the upregulated genes show an enrichment for pathways involved in neuronal development and differentiation (Fig 1). Using SLAM-seq, a method to identify newly transcribed genes, the authors demonstrated that pro-neural genes such as *NEUROD1* and *INSM1* were upregulated almost immediately following LSD1i, as were components of the BMP signaling pathway. Interestingly, a similar set of genes and pathways upregulated by LSD1i were found by Park *et al*.

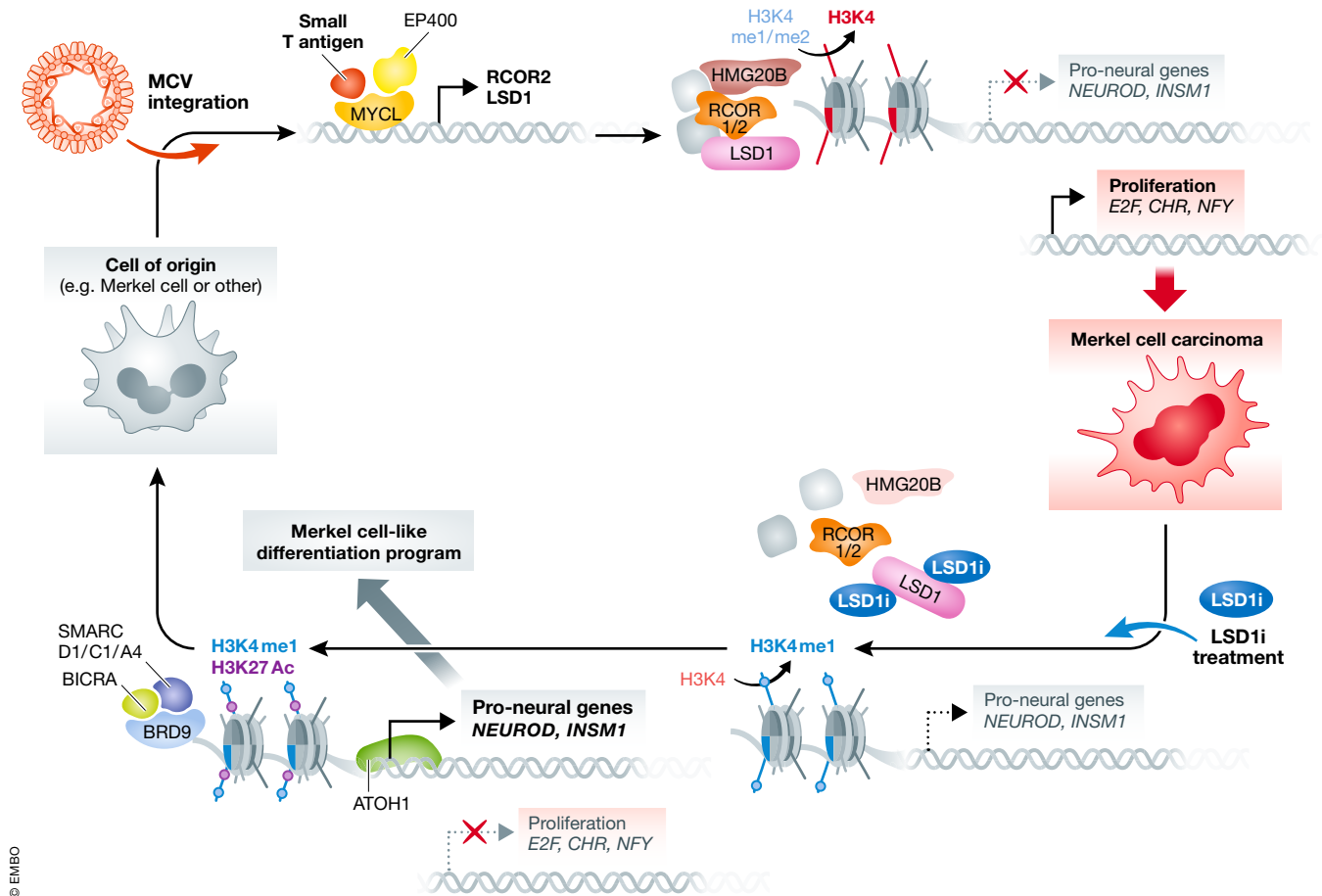
By comparing the genes upregulated by LSD1i in MCCs to the genes naturally expressed by normal Merkel cells, Leiendecker *et al* suggested that LSD1i promoted the differentiation of MCC into Merkel-like cells. Consistent with this notion, using ChIP-seq, Park *et al* found that LSD1 and its binding partners compete with *ATOH1*, a master regulator of Merkel cell fate, for binding to an overlapping set of genes, further

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**Figure 1. Inhibition of LSD1 impairs MCC transcriptional program.**

After MCV integration, the small T antigen forms a complex with MYCL and EP400, driving the expression of LSD1 and other CoREST complex members. The LSD1-CoREST complex mediates oncogenic transformation by repressing pro-neural genes through direct removal of H3K4 mono-/dimethyl marks, while indirectly promoting tumor cell proliferation. LSD1i treatment destabilizes the LSD1-CoREST complex through the degradation of HMG20B. The resulting changes in Histone marks deposition allow the ncBAF complex to recognize the H3K27 acetylation marks through BRD9 and mediate the expression of pro-differentiation genes, also with ATOH1 activity, restoring a Merkel cell-like differentiation program.

supporting the idea that LSD1 competes for Atoh1 binding sites and represses the normal Merkel cell transcriptional program.

The two groups dissected in more detail the molecular mechanisms by which LSD1 sustains MCC tumor phenotype. By performing immunoprecipitation followed by mass spectrometry analysis, the authors of both groups showed that LSD1 interacts with members of the CoREST repressor complex including the core members HDAC1/2, the scaffolding proteins RCOR1-3, and several non-canonical components such as HMG20A/B. Leiendecker *et al* demonstrated that LSD1i leads to the loss of HMG20B binding, thereby impairing the assembly and stability of the LSD1-CoREST complex. Supporting the notion that the loss of

HMG20B from this complex is important for the therapeutic effect of LSD1i, knockdown of HMG20B also impaired MCC cell growth. Interestingly, a short pulse of LSD1i was sufficient to promote long-term increase of pro-neural gene expression and disassembly of the LSD1-CoREST complex, leading to sustained pro-neural differentiation and loss of tumorigenic potential.

Park *et al* performed a genome-wide CRISPR screen to identify genes that would be positively or negatively selected upon LSD1i treatment. The level of histone lysine methylation marks is regulated in a coordinated manner by the activity of methyltransferases (“writers”) and demethylases (“erasers”, like LSD1). But the effect of such modifications depends on chromatin effector

molecules (“readers”) that can recognize those marks and mediate chromatin remodeling and the associated transcriptional changes (Hyun *et al*, 2017). The screen of Park *et al* showed that several components of the non-canonical BAF (ncBAF) chromatin-remodeling complex (“reader”), including BRD9, were positively selected upon LSD1 inhibition, indicating that they are mediating the downstream effects of LSD1i. Park *et al* demonstrated that BRD9 and the ncBAF complex compete with LSD1 for binding to overlapping target genes, and that BRD9 mediates chromatin opening and the transcriptional activation of LSD1 targets after LSD1i treatment.

In conclusion, these two studies elegantly demonstrate that pharmacological LSD1

inhibition is very efficient to promote the differentiation of MCCs into normal Merkel-like cells strongly impairing MCC growth. These results show that in addition to leukemia, a subtype of colorectal cancer and basal cell carcinoma (Sanchez-Danes *et al*, 2018; de The, 2018), therapies that induce differentiation could be very effective in the treatment of solid tumors. Epigenetic regulators targeting could be very efficient to achieve this goal, maximizing therapeutic benefits and minimizing side effects.

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