An epigenetic switch: from senescent melanocytes to malignant melanoma (and back)

Verena Wagner[1,2] and Jesús Gil[1, 2,*]

[1]MRC London Institute of Medical Sciences (LMS), Du Cane Road, London, W12 0NN, UK.

[2]Institute of Clinical Sciences (ICS), Faculty of Medicine, Imperial College London, Du Cane Road, London W12 0NN, UK.

*Correspondence: jesus.gil@imperial.ac.uk

Oncogene-induced senescence is an important barrier during melanomagenesis. In this issue of *Cancer Cell,* Yu et al. show how elevated expression of structurally-unrelated H3K9 demethylases disables senescence and constitutes a liability that can be exploited to restore senescence in melanoma by pharmacological inhibition of these epigenetic regulators.

Oncogene-induced senescence (OIS) was first described in 1997 when Serrano et al. showed that expression of oncogenic Ras led to premature senescence in MEFs and human fibroblasts (Serrano et al., 1997). OIS is characterized by a stable growth arrest, depending on the engagement of the $p16^{INK4a}$/Rb and p53 tumor suppressor pathways, and other distinctive changes such as chromatin reorganization or the acquisition of a senescence-associated secretory phenotype (SASP). Once implemented, OIS prevents proliferation of cells harbouring dangerous mutations and constitutes a barrier against tumor development. The prototypic example of benign lesions enriched in senescent cells are melanocytic nevi harbouring Braf oncogenic mutations (Michaloglou et al., 2005). Although benign nevi can persist in the skin for years, some eventually progress and give rise to melanomas. For pre-malignant lesions to develop into more advanced tumors, overcoming senescence is a general prerequisite. Therefore, mechanisms that allow bypass of senescence are of great interest to understand cancer progression. Loss of function mutations of key tumor suppressor genes (that also control senescence) like *TP53* or *CDKN2A* are the most prominent examples applicable to many tumor types. However, the mechanisms facilitating senescence bypass during nevus-to-melanoma transition remain unclear, as p53 mutations are rare in melanoma and $p16^{INK4a}$ deficiency alone fails to abrogate melanocyte senescence. Alternative mechanisms such as PTEN depletion (Vredeveld et al., 2012) have been suggested to explain how senescence is bypassed during melanomagenesis.

In this issue of *Cancer Cell*, Yu et al. (2018), propose a new mechanism to explain how a subgroup of melanomas bypasses senescence. Using primary human melanocytes, mouse and zebrafish models, Yu et al. (2018) demonstrate that H3K9 demethylation by the enzymes

LSD1 and JMJD2C can transform senescent melanocytes into malignant tumor cells. LSD1 and JMJD2C activity can thereby both prevent senescence induced by oncogenic Ras or Braf and, even more importantly, enable melanocytes to overcome senescence and re-enter the cell cycle as malignant cells. The authors show that overexpression of LSD1 or JMJD2C in combination with oncogenic Braf results in aggressive melanomas with distant metastases in lymph nodes and lungs, which provides the basis for a new mouse model of metastatic melanoma. The authors identify that the expression of LSD1, JMJD2C and other Histone 3 Lysine 9 (H3K9) demethylases is elevated in a relevant subset of malignant melanoma cell lines as well as primary patient samples, while it is barely detectable in melanocytic nevi. Of note, high expression levels of LSD1 and JmjC family members are associated with reduced overall survival and increased tumor cell proliferation. As both enzymes are overexpressed not only in melanoma but also in a variety of cancers (Berry and Janknecht, 2013) these findings might as well be relevant for other entities.

Epigenetic control is an important regulatory layer during senescence. The best example is how Histone 3 Lysine 27 (H3K27) tri-methylation by Polycomb group proteins represses the *CDKN2A* locus in proliferating cells, while activation of Ras induces its demethylation by JMJD3 resulting in the expression of $p16^{INK4a}$, a key mediator of oncogene-induced senescence (Barradas et al., 2009). Another prominent alteration observed in cells undergoing OIS is the reorganization of H3K9me3 chromatin in senescence-associated heterochromatin foci (SAHFs). This results in the repression of E2F-dependent genes, contributing to the stability of the senescence arrest (Narita et al., 2003). This is, at least in part, carried out by the Histone-lysine N-methyltransferase SUV39H1, which can initiate heterochromatin formation by H3K9 tri-methylation (Figure 1A). The relevance of SUV39H1 for senescence is highlighted by the fact that SUV39H1 deficiency in mice prevents Ras-induced senescence in lymphocytes leading to formation of lymphoma (Braig et al., 2005). The important role of the H3K9 methylation status for the regulation of senescence and tumorigenesis is further underlined in the present study. Overexpression of LSD1 or JMJD2C causes loss of H3K9me3 at E2F target promotors allowing for transcriptional activation of genes promoting G1 to S

phase transition. The authors also express a histone 3 mutant that cannot be methylated at lysine 9 (substitution of lysine 9 by an arginine residue). Interestingly, inducible overexpression of JMJD2C or the histone 3 mutant was sufficient to reinstate proliferation in already senescent MEFs. This shows the dynamic nature of epigenetic regulation and heterochromatin formation: H3K9 tri-methylation is not an irreversible mark but its methylation status can be changed by demethylases or histone turnover even after senescence induction.

Strategies targeting senescence reactivation, also referred as 'pro-senescence' therapies, are promising to control tumorigenesis (Acosta and Gil, 2012). Since chromatin modifiers are attractive therapeutic targets, Yu et al. (2018) aimed to address whether inhibition of LSD1 or JMJD2C can restore senescence in melanoma cells. Treatment of Ras/LSD1 overexpressing cells with two different inhibitors of LSD1 or transduction of an LSD1 shRNA led to an increase in H3K9me3 and restored senescence as assessed by increased SA-ß-Gal activity and impaired cell proliferation (Figure 1B). Similar results were observed for JMJD2C. These small molecules were also tested in mouse and human melanoma cells lines as well as in primary patient melanoma cells. Interestingly, cell lines previously assigned to the "H3K9 demethylases high" group, but not cell lines with relatively low expression of H3K9 demethylases, also showed a senescence response characterized by proliferation arrest, high SA-ß-Gal activity and an increased SASP. This suggests that these cells rely on H3K9 demethylases for the prevention of senescence. Finally, the authors confirm these findings *in vivo* using the B16F10 cell line in immunocompetent mice as well as xenograft models with melanoma cell lines and patient-derived cells. 2-PCPA-1a, a small molecule inhibitor of LSD1, as well as IOX1, an inhibitor of JMJD2 family members, induced senescence and restored H3K9 tri-methylation in all models that displayed high expression levels of H3K9 demethylases. Interestingly, this was associated with infiltration of $CD11b^+$ innate immune cells, what could result in the immune-mediated elimination of senescent cells. This is noteworthy because the senescent cells present in melanocytic nevus do not seem to elicitate the immune-mediated surveillance that is characteristic in other tissues such as liver.

Taken together, Yu et al. (2018) discover a role for the H3K9 demethylases LSD1 and JMJD2C as major drivers of melanomagenesis by enabling melanocytes to overcome senescence. The reliance on these chromatin modifiers also constitutes a therapeutic liability. Pharmacological inhibition of LSD1 or JMJD2C can restore senescence in melanoma cells with elevated expression of these enzymes, therefore providing a novel strategy to treat a subset of melanoma patients. Small molecule inhibitors of H3K9 demethylases could potentially be used in combination with Braf inhibitors, or alone in the case of primary or secondary resistance to Braf inhibitors (Haferkamp et al., 2013) . As senescence induction in melanoma cells led to the infiltration of immune cells *in vivo*, combination effects with immune checkpoint inhibitors should be further investigated in the future.

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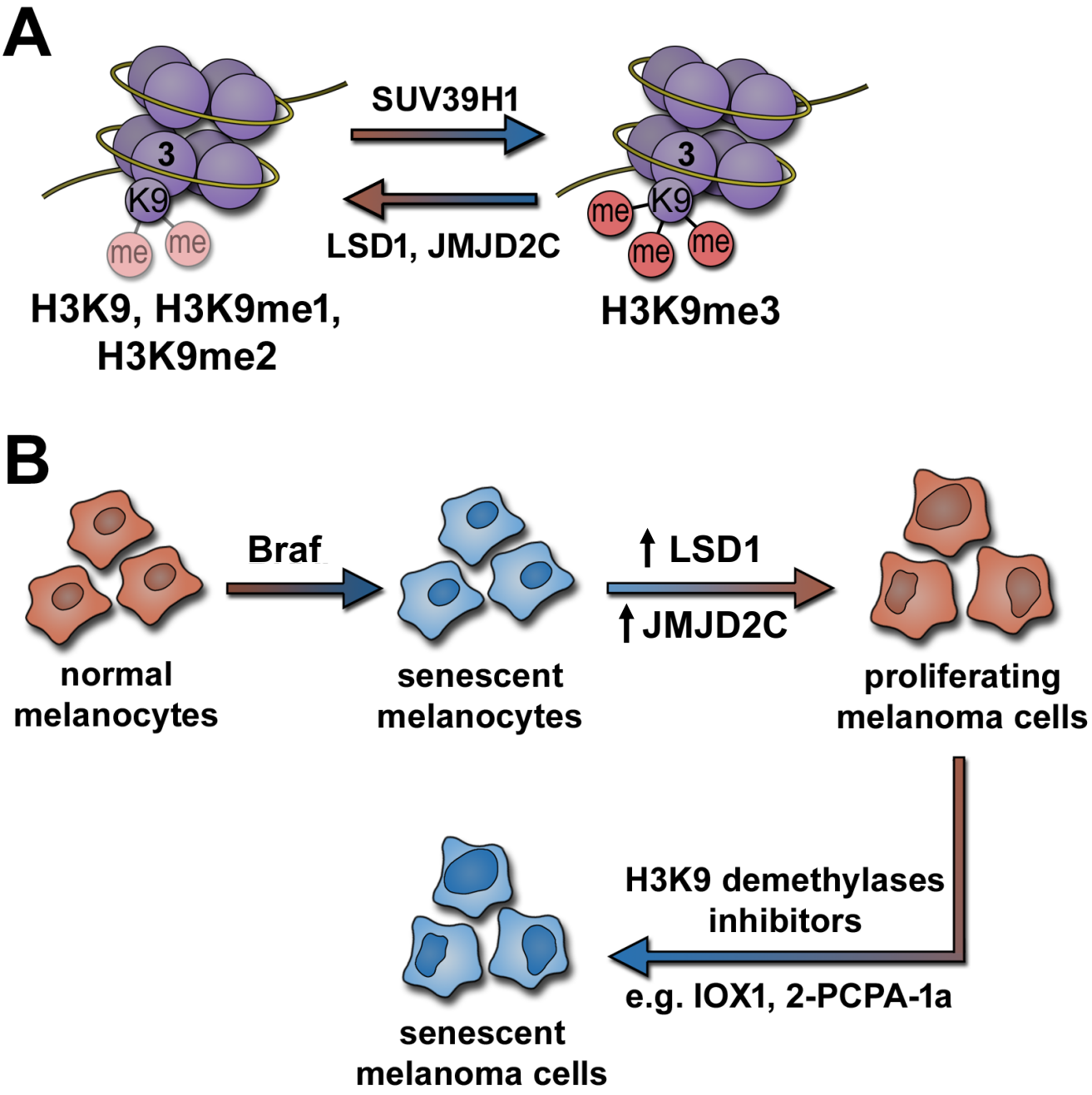
FIGURE LEGEND

Figure 1. Re-engaging senescence in melanoma by targeting H3K9 demethylases.

(A) Histone 3 Lysine 9 (H3K9) tri-methylation by Histone-lysine N-methyltransferase SUV39H1 is a feature of senescence leading to silencing of E2F target genes and heterochromatin formation. H3K9 demethylases like LSD1 and JMJD2C can reverse this and prevent senescence or reinstate proliferation in senescent cells. (B) Overexpression of H3K9 demethylases leads to malignant transformation of senescent melanocytes. In melanoma cells with elevated expression of these enzymes, senescence can be re-induced by shRNA-mediated knockdown or pharmacological inhibition of LSD1 or JMJD2C.


A
SUV39H1
LSD1, JMJD2C
3
K9
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H3K9, H3K9me1,
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H3K9me3
B
Braf
LSD1
JMJD2C
normal
melanocytes
senescent
melanocytes
proliferating
melanoma cells
H3K9 demethylases
inhibitors
e.g. IOX1, 2-PCPA-1a
senescent
melanoma cells