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Induction of senescence in melanoma: thinking outside the cell

Narendra Wajapeyee and Michael R. Green

In response to genotoxic stress, such as telomere erosion or oncogenic stimuli, primary human cells undergo a growth arrest program known as cellular senescence. Senescence is thought to be a barrier to tumorigenesis that prevents the proliferation of cells at risk for neo-plastic transformation. Cancer cells acquire genetic and epigenetic changes that allow them to bypass senescence and to proliferate indefinitely.

Although growth-arrested, senescent cells remain metabolically active and secrete a myriad of factors including cytokines, growth factors, and proteinases, a phenomenon that has been dubbed the secretory-associated senescence phenotype (SASP) (Coppé et al., 2010) or the senescence messaging secretome (Kuilman and Peeper, 2009). Some of these secreted proteins work to induce or reinforce the senescence program. For example, melanocytes that are induced to undergo senescence by expression of an activated *BRAF* oncogene synthesize and secrete insulin-like growth factor binding protein 7 (IGFBP7), which promotes growth arrest through an autocrine/paracrine mechanism (Wajapeyee et al., 2008). However, other factors secreted by senescent cells are associated with inflammation and malignancy and, paradoxically, can promote tumorigenesis.

In a recent study, Ohanna et al. sought to determine whether senescent melanoma cells, like senescent primary cells, have an SASP. Toward this end, the authors induced senescence in the human melanoma cell line 501mel by siRNA-mediated knockdown of microphthalmia-associated transcription factor (MITF), a melanocyte-specific factor that can function as an oncogene in melanoma. Expression profiling of MITF knockdown versus control 501mel cells revealed upregulation of a suite of genes encoding secreted proteins, many of which were known SASP components. Interestingly, as in *BRAF*-induced senescent melanocytes (Wajapeyee et al., 2008), the senescent 501mel melanoma cells also secreted IGFBP7.

One of the most highly upregulated genes found by Ohanna et al. encoded the secreted protein chemokine ligand-2 (CCL2). Increased secretion of CCL2 was confirmed in the conditioned media (CM) of senescent melanoma cells relative to control cells. Treatment of naïve 501mel cells with CM collected from the senescent 501mel cells promoted pro-invasive behavior. Similarly, naïve melanoma cells treated with recombinant CCL2 (rCCL2) also displayed a proinvasive phenotype, indicating a critical role for CCL2 in the secretome.

To confirm these in vitro results, Ohanna et al. turned to a xenograft mouse model. 501mel cells normally do not form xenografts in athymic nude mice. However, 501mel cells treated with either CM from senescent 501mel cells or rCCL2 formed tumors as well as lung

metastases, indicating that CCL2 alone is sufficient to promote both tumorigenesis and metastasis.

Previous studies have shown that expression of many SASP components depends on the transcription factor NF- κ B, whose activity is increased during senescence induction (Coppé et al., 2010). Ohanna et al. found that knockdown of MITF in 501mel cells induced NF- κ B activity. Moreover, immunohisto-chemical analysis of human melanoma samples revealed an inverse relationship between MITF and nuclear NF- κ B staining. Most importantly, inhibition of NF- κ B prevented CCL2 upregulation and consequently its secretion, thereby preventing the pro-invasive effects of CM from senescent 501mel cells.

Previous studies have shown that components of the DNA damage response are required for the increased secretion of some SASP factors (Coppé et al., 2010). This prompted the authors to test the role of poly (ADP-ribose) polymerase 1 (PARP-1), a genotoxic sensor involved in DNA repair and known to activate NF- κ B. They found that knockdown of MITF in 501mel cells led to an increase in PARP-1 activity, which was required for NF- κ B activation (Figure 1). Based upon these collective results, the authors named this senescence program as the PARP-1- and NF- κ B-associated secretome (PNAS).

Interestingly, the PNAS is not restricted to MITF-knockdown 501mel cells. For example, other senescence-inducing stimuli, such as oxidative stress (hydrogen peroxide) or treatment with chemotherapeutic drugs (such as temozolomide and fotemustine), also led to activation of the PNAS, secretion of CCL2, and promotion of a pro-invasive phenotype in 501mel cells in vitro. An important, unresolved question that would bolster the relevance of this finding is whether chemotherapeutic drug treatment also promotes tumor growth in the mouse xenograft model. Furthermore, whether this phenomenon extends to other melanoma cell lines remains to be determined. Interestingly, however, the authors found that a similar secretory pathway may exist in the breast cancer cell line MCF7, suggesting the PNAS may be of general importance for other cancer types as well.

Arguably, the most unexpected finding of Ohanna et al. is that, despite the complexity of the secretome, a single secreted protein, CCL2, is able to drive both tumorigenesis and metastasis, which are complex biological processes that require multiple genetic and epigenetic aberrations. Even more remarkable is the finding that transient exposure to CCL2 is sufficient to reprogram 501mel cells permanently, altering their tumorigenic and metastatic properties. How is this possible? Although not addressed in this study, an attractive model is that transient exposure to CCL2 induces transcription of the endogenous *CCL2* gene (presumably through NF- κ B), thereby establishing a positive autoregulatory feedback loop (Figure 1). A precedent for this idea is that IL-1 α , another SASP component, regulates its own synthesis in an autocrine, receptor-mediated, positive-feedback loop via NF- κ B (Niu et al., 2004).

The results of Ohanna et al. have implications for the treatment of melanoma and other cancers. A variety of cancer cell types, including melanoma cells, can be induced to undergo senescence. In fact, it is now recognized that standard chemotherapy and ionizing radiation not only induce an expected apoptotic response but can also trigger senescence induction

(Nardella et al., 2011). Reactivation of a senescence program therefore represents a potential therapeutic strategy for the treatment of melanoma, whose resistance to more traditional therapies – notably, those that induce apoptosis – limits the efficacy of current anti-melanoma drugs.

However, a potential problem of senescence induction as a therapeutic strategy is that the senescent cells will secrete proteins that promote the tumorigenic properties of any neighboring transformed cells that have not undergone senescence or apoptosis. The results of Ohanna et al. hint at a potential solution to this conundrum. Targeted inhibition of the PNAS using, for example, NF- κ B or PARP-1 inhibitors in conjunction with traditional chemotherapy may prevent the adverse consequence of senescence induction (Figure 1).

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Figure 1.

A PARP-1- and NF- κ B-dependent senescence-associated secretome promotes melanoma tumorigenesis and metastasis. RNAi-mediated knockdown of MITF, or exposure to other senescence-inducing stimuli, leads to induction of a PARP-1- and NF- κ B-associated secretome. The secretome, mainly through CCL2, can exert pro-tumorigenic and prometastatic effects on neighboring cells and the micro-environment. Transient exposure to CCL2 can permanently alter the tumorigenic and metastatic properties of melanoma cells, which may involve a positive autoregulatory feedback loop that induces transcription of the endogenous *CCL2* gene, most likely through NF- κ B (dashed line). Targeted inhibition of the PNAS using, for example, PARP-1 or NF- κ B inhibitors in conjunction with traditional chemotherapy may prevent the pro-tumorigenic and prometastatic effects of senescence induction, thereby improving therapeutic outcome.