A Role for CXCR2 in Senescence, but What about in Cancer?

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Abstract

Senescence is an irreversible arrest triggered by stresses such as telomere shortening, DNA damage, or oncogenic signaling. Oncogene-induced senescence occurs in preneoplastic lesions, but it is absent from full-blown malignancies suggesting a tumor suppressor function. We recently found that depletion of the receptor CXCR2 [which binds to chemokines such as interleukin (IL)-8 or GROα] delays both replicative senescence and impairs the senescence response to oncogenic signals. Our findings suggest that signaling by IL-8 and GROα might limit tumor growth by reinforcing senescence early in tumorigenesis. The challenge remains in how to integrate this with the well-known tumor promoting effects of IL-8 and GROα.

Senescent Cells Produce Multiple Secreted Factors

Senescence was first described as the irreversible growth arrest reached by human diploid fibroblasts (HDF) after serial passage in culture (1). Subsequently, different insults have been shown to induce a similar response in cultured cells. The most relevant among them is aberrant oncogenic signaling that triggers what has been defined as oncogene-induced senescence (OIS). Evidence for a physiologic role for senescence has accumulated and cellular senescence seems to act as a tumor barrier against potentially dangerous mutations in vivo. This is exemplified by the manifestations of OIS in benign and premalignant lesions initiated by single activated oncogenes. A number of benign and premalignant lesions such as melanocytic nevi, lung adenomas, dermal neurofibromas, and prostate intraepithelial neoplasias among others, are enriched in senescent cells, highlighting the relevance of senescence as a mechanism to prevent cancer progression (2).

The hallmark of senescent cells is their resistance to re-entry into cell cycle despite stimulation with growth factors. Senescent cells are metabolically active and display changes in cell morphology, physiology, and gene expression. In addition, senescent cells undergo active changes in chromatin organization essential to their growth arrest. For example, senescent cells display characteristic heterochromatic foci (termed senescence-associated heterochromatin foci) enriched in repressive marks such as trimethylated lysine 9 of Histone H3 (H3 K9me3) that are required for silencing E2F-target genes (3). In addition, senescence is linked to the activation of the DNA damage response. Senescence-associated DNA damage-foci constitute another of the hallmarks of senescent cells (4). The activation of p53 by the DNA damage response contributes to establishing the arrest. Among multiple changes in gene expression, senescent cells up-regulate the transcription of cyclin-dependent kinase inhibitors such as p21<sup>CDIP1</sup> or p16<sup>INK4a</sup>. Besides these, senescent cells up-regulate the transcription of multiple secreted factors (5–7). Senescent cells secrete a complex cocktail that it is referred as the senescent-associated secretome or senescent associated secretory phenotype. Among these secreted molecules, there are extracellular proteases [urokinase-type plasminogen activator (uPA), tPA], matrix components (MMP), growth factors, proinflammatory cytokines [interleukin (IL)-6, IL-1α, IL-1β], and chemokines (IL-8, GROα, MCP-1). The up-regulation of secreted proteins is observed in cells undergoing replicative senescence or OIS regardless of their origin, either epithelial, fibroblast, or endothelial (5, 7, 8). This senescent-associated secretory phenotype can trigger different and, in some cases, opposing effects in the microenvironment and other surrounding cells. Work by Judith Campisi’s group (9) suggests that factors secreted by senescent fibroblasts promote cancer progression. More specifically, secreted MMP3 can affect the differentiation of epithelial cells (10), and vascular endothelial growth factor secreted by senescent fibroblast can promote angiogenesis (11).

On the other hand, several groups have reported a tumor suppressive role for some of the factors secreted by senescent cells. Work from Rene Bernard shows that plasminogen activator inhibitor (PAI)-1 is necessary and sufficient for the induction of senescence (12). PAI-1 is up-regulated during senescence and acts by inhibiting the uPA-mediated activation of the AKT pathway (12). More recently, Michael Green’s group (6) showed that IGFBP7 (also known as MAC25), a factor previously identified as up-regulated during serial passage of HMECs, mediates senescence induced by oncogenic BRAF (13). Importantly, the IGFBP7 promoter is methylated in a large number of tumors, and injection of IGFBP7 in BRAF melanoma xenografts suppressed tumors, indicating the central role of this secreted protein in determining cellular fate in response to BRAF expression.

Proinflammatory cytokines and chemokines secreted by senescent cells also regulate the senescent growth arrest (6, 7) and trigger an innate immune response that results in clearance of senescent lesions (14). Kullman and colleagues observed that IL-6 is up-regulated during OIS, and that knockdown of IL-6 or its receptor bypasses OIS induced by oncogenic BRAF. The expression of IL-8 is also induced during OIS, and depletion of IL-8 expression resulted in similar effects as to depletion of IL-6. Importantly, and in agreement with our findings (6), high levels of IL-8 expression are observed in the nonproliferative crypts of colon adenomas (7), suggesting that these cytokines and chemokines function in vivo to promote or maintain senescence of benign human tumors.
Reinforcement of Senescence by CXCR2-Binding Chemokines

In a loss-of-function screen, our group identified an shRNA targeting the chemokine receptor CXCR2 (IL8RB) as able to bypass senescence in HDFs (6). The CXCR2 receptor is activated by CXC chemokines such as IL-8, CXCL1, CXCL2 and CXCL3 (GROα, β, and γ), CXCL5 (ENA-78), CXCL6 (GCP2), and CXCL7 (NAP2). The expression of all of these factors is strongly induced during OIS in an NF-κB and CAAT/enhancer binding protein–β-dependent manner. Moreover, the expression of the CXCR2 receptor also increases upon OIS and during serial passage of HDFs. This coordinated up-regulation of the ligands and the receptor is probably relevant in implementing senescence. Inhibition of CXCR2 signaling, either interfering with CXCR2 or their ligands, alleviates OIS, whereas the depletion of IL-8 and GROα by RNA interference, or by using neutralizing antibodies also has similar effects, which correlate with CXCR2 activity. Conversely, the overexpression of CXCR2 or its paralogue CXCR1 in different cell types causes premature senescence. Importantly, senescence induced by CXCR2 is dependent of p53 activity as p53-null MEFs do not undergo senescence upon overexpression of CXCR2 but continue proliferating. Supporting these data, we showed that knockdown of CXCR2 significantly diminished the DNA-damage response (6).

To probe whether the role of CXCR2 in mediating senescence has physiologic implications, we analyzed CXCR2 expression in preneoplastic lesions. First, we assessed the expression of CXCR2 and CXCR1 in samples of prostate intraepithelial neoplasia, a precursor lesion for prostate cancer enriched in senescent cells (15). Levels of both receptors were elevated in prostate intraepithelial neoplasia when compared with normal or atrophic glands of the same patients. Although less dramatic, the levels of CXCR2 and CXCR1 were reduced when comparing prostate cancer and prostate intraepithelial neoplasia lesions. In addition, we analyzed the expression of CXCR2 in papillomas induced upon treatment with 7,12-dimethylbenz[a]anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA) on mice. Previously, these papillomas have been shown to be enriched in senescent cells (5). The expression not only of CXCR2 but also of their ligands was

![Figure 1](https://cancerres.aacrjournals.org)
A Tumor Suppressor Role for Chemokine Signaling

The connection between inflammation and cancer was first noted by Virchow in the 19th century (16). Chronic inflammation is a prerequisite for the development of some tumor types, and immune cells are found infiltrated in diverse tumor lesions. The overall view that inflammation has multiple tumor-promoting effects is well established. More specifically, proinflammatory cytokines, such as IL-6, IL-8, or GROα contribute to multiple protumorigenic activities. For example, IL-6 and IL-8 are necessary mediators of the effects of Ras in tumorigenesis (17, 18). Therefore, these observations need to be integrated with the possible tumor suppressive effects of IL-8 or GROα.

The idea that senescence (and specially, OIS) operates as an intrinsic tumor suppressor mechanism has gained strength with evidence for its role in vivo. As a general consideration, for tumors to progress, additional mutations must occur to cancel the senescent arrest elicited in the benign lesions. Our results suggest that chemokine signaling reinforces senescence. In addition to that intrinsic role, CXCR2 ligands can also control tumor growth by recruiting the innate immune system. For example, the reactivation of p53 in hepatocellular carcinoma induces senescence and triggers the up-regulation of diverse proinflammatory cytokines (i.e., GROα), which activate an innate immune response. In this context, the recruitment of macrophages, neutrophils, and natural killer cells is responsible for tumor cell clearance (14). Taken together (the reinforcement of senescence and immune-mediated clearance of incipient tumors), the relevance of the tumor suppressive effects of proinflammatory chemokines gains strength. Therefore, the assumption could be that mutations or alterations disrupting the tumor suppressive action of CXCR2 signaling will facilitate tumor progression. This would be relevant in a context where senescence is important, such as in preneoplastic lesions or in response to chemotherapy. We can think of at least three alternative mechanisms to neutralize CXCR2 prosenescence activity: alterations in pathways downstream of CXCR2, down-regulation of CXCR2 expression, or mutations affecting CXCR2 activity (summarized in Fig. 1).

As senescence induced by CXCR2 relies on p53, mutations interfering with that pathway will cancel the prosenescence action of CXCR2. This is exemplified by experiments comparing the effect of CXCR2 in wild-type or p53-null MEFs (6). The coexistence of a CXCR2 prosenescence activity and its role in tumor growth (19) can be reconciled with mutations on the p53 pathway and mirror the effect on other context-dependent oncogenes. Indeed, these mutations, alone or in combination with other alterations, may switch CXCR2 from prosenescence to protumorigenic. Although paradoxical, similar mechanisms explain how other genes, such as Ras or transforming growth factor-β, can have seemingly opposite effects on tumor growth depending on genetic context.

In addition, our work showed that levels of CXCR2 are up-regulated in preneoplastic lesions, and evidence suggests that its expression might subsequently decrease during cancer progression, as seems to be the case in prostate cancer. That is consistent with the idea that high CXCR2 levels are associated with senescence in premalignant lesions and loss of CXCR2 expression contributes to reduce the severity of the arrest. Interestingly, the decrease in the expression of CXCR2 as the tumor progresses can proceed while the levels of CXCR2 ligands still remain high and probably drive tumor progression through their paracrine actions. In this regard, low levels of CXCR2 in head and squamous cell carcinoma coexist with increased expression of CXCR2 ligands (20). We also found evidence for a direct correlation between CXCR2 expression and survival, whereas IL-8 expression inversely correlates with survival in breast cancer.1

Finally, mutations in CXCR2 itself could impair its ability to induce senescence. As mentioned above, we found one inactivating mutation in CXCR2 in the NCI-H1395 cell line. Although a more detailed study must be performed to evaluate the incidence of mutations, the presence of one mutation is, in itself, interesting. Despite the functional effects associated with the allele, caution must be taken as it might well be either a passenger mutation acquired late in tumorigenesis, or even in tissue culture. However, its coexistence with an activated BRAF allele and the functional experiments that we conducted suggest otherwise.

In summary, the actions exerted by CXCR2 ligands during tumor progression are complex. Although our evidence suggests a clear prosenescence effect in primary cells, they can also exert multiple protumorigenic actions during tumor progression. In the end, their effect will depend on the stage and status of the lesion, its origin, and crucially, its genetic background. Performing studies in defined animal models in which the effect of CXCR2 signaling on the senescent response can be dissected will be necessary to understand the complex contribution of CXCR2 signaling to tumor progression, and whether their prosenescence effects can be manipulated for therapeutic advantage.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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1 Unpublished observations.
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