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AUTHOR'S VIEWS

Tumor growth fueled by spurious senescence phenotypes

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ABSTRACT

Cancer treatments can induce a form of senescence that halts cellular division while allowing continued secretion of tumor-promoting proteins. We recently found that antiangiogenic treatment resistance can lead to a transient hijacking of the senescence-controlled secretory machinery that, when therapeutically targeted during treatment cessation, can blunt rebound tumor growth.

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Senescence is a normal part of the aging process and classically defined as a cell that is no longer able to divide but remains alive, albeit in an altered state. A senescent cell can protect tissues from acute damage and aid in repair, but also have negative effects, such as sending nefarious secretory signals to neighboring cells that promote inflammation and tumor growth.[1] Induction of senescence-associated secretory phenotypes (SASPs) can be caused by *intrinsic* changes (i.e., by telomere shortening, oncogenes, etc.) as well as via numerous *extrinsic* stimuli that include, perhaps counterintuitively, cancer treatments. Radiation, chemotherapy, and several molecular targeted agents can induce senescence; however, these effects depend on the treatment type, dose, and duration, amongst several other factors. Methods to detect senescence cell positivity include senescence-associated (SA) cellular hallmarks such as heterochromatin foci (SAHF), SASP, β-galactosidase (SA-β-gal), hypertrophy, and/or expression changes of growth arrest regulators such as tumor protein p53 (TP53, best known as p53)/p21 or p16/retinoblastoma protein (pRb) (and others). But markers can vary between cell and drug type, and it remains unclear whether senescence always includes terminal cell differentiation (see[2] for review). Indeed, there are increasing clues that suggest treatment can induce spurious uncommitted phenotypes – variously called 'pseudosenescent', 'premature', 'accelerated', or 'senescence-like'[3] – that represent an atypical senescent cell capable of 'escaping' permanent growth arrest.[2] This has raised the question as to whether SASP-like phenotypes induced by therapy are capable of driving tumor/stromal interactions that, in turn, can fuel tumor growth and metastatic spread.[1]

Antiangiogenic therapy aims to disrupt blood vessel formation that is critical for the growth and dissemination of tumor cells. The vascular endothelial growth factor (VEGF) has been the primary target for several clinically approved drugs in the metastatic setting; however, initial efficacy is undermined by resistance for the majority of patients.[4] In a paper recently published in *Cell Reports*, we undertook a series of studies to investigate the role of senescence in promoting tumor growth after antiangiogenic therapy.[5] This was done for three reasons. **First**, angiogenesis inhibitors have been reported to promote metastasis in certain preclinical settings.[6] While the clinical implications of such findings remain unclear, it is possible that these effects may be most consequential after therapy has stopped – something that occurs in patients after treatment failure or toxicity.[7] **Second**, VEGF pathway disruption has also been shown to induce senescence phenotypes in both clinical and preclinical studies,[8] but the durability of these cellular changes remain understudied. **Third**, VEGF pathway inhibition can induce a broad array of SASP-like circulating cytokine changes in patients. These systemic blood-protein changes have been investigated as surrogate biomarkers of response, but may also be contributors to post-treatment disease progression in patients who have relapsed.[9]

In our studies, we evaluated senescence markers and tumor growth after resistance to clinically approved VEGF receptor tyrosine kinase inhibitors (VEGFR TKIs), and again after treatment had been stopped for short-term (24–48 hrs) or long-term (2–6 months) periods.[5] We found that VEGFR TKI-resistant mouse and human tumor cells originating from kidney carcinoma, breast carcinoma, or melanoma, progressed much more rapidly than controls when implanted orthotopically into mice when treatment was halted (representing short-term withdrawal conditions). Interestingly, these withdrawal-mediated tumor growth rebounds were found to diminish following long-term periods of drug removal, suggesting that tumor-promoting phenotypes were reversible for most cells. We next examined nine tumor and non-tumor cell lines resistant to two VEGFR TKIs (sunitinib or axitinib) and found all were positive for senescence markers that included increased cell size, p21 upregulation, and SA-β-gal. However, these markers were inconsistent amongst the cell lines and all ultimately diminished following protracted periods of drug removal. In subsequent experiments, we utilized 5-dodecanoylaminofluorescein di-β-D-galactopyranoside (C_{12}

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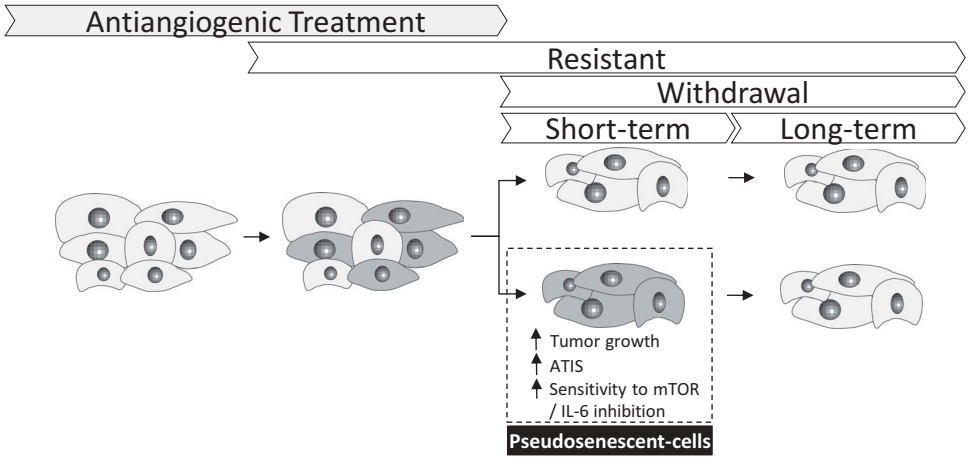

Figure 1. Emergent tumor-promoting properties in antiangiogenic therapy-resistant cells. Proposed model of persistent pseudosenescent phenotypes in cells during short-periods of withdrawal after resistance to antiangiogenic VEGFR TKI treatment. Resistance increases senescence marker-positive cells (dark grey) that have hijacked the tumor-promoting senescence-associated secretory machinery but that ultimately reverse after long-term withdrawal. Targeting mTOR and IL-6 can blunt withdrawal-mediated rebound growth. *ATIS, antiangiogenic treatment-induced secretome; VEGFR, vascular endothelial growth factor receptor; TKI, tyrosine kinase inhibitor; IL-6, interleukin-6; mTOR, mammalian target of rapamycin.*

FDG), a fluorogenic substrate for β-gal activity to identify live senescent marker-positive cells.[10] Using flow cytometric sorting, we found that $C_{12}FDG^{+}$ (high SA-β-gal expressing) cell populations had increased growth rates *in vitro* and *in vivo* compared to $C_{12}FDG^{-}$ (low SA-β-gal expressing) cells. Finally, using transcriptomic and protein analysis, we observed that VEGFR TKI-resistant cells had an SASP-mimicking antiangiogenic therapy induced secretome (ATIS) that could persist during short-term treatment withdrawal periods. We found the ATIS was maintained after short treatment gaps and coincided with tumor growth accelerations following implantation into mice. Critically, our results showed that therapeutic targeting of mammalian target of rapamycin (mTOR) and interleukin 6 (IL-6) – both regulators of the SASP – could limit withdrawal-mediated tumor growth (Figure 1).

Taken together, these studies suggest that there is a short 'window' immediately after withdrawal of treatment after VEGFR TKI resistance where tumor growth is promoted by sub-populations of cells enriched for senescence marker positivity. These transient pseudosenescent cells have reversible secretory profiles closely associated with cellular aging. It is possible that senescence-targeted treatment strategies – particularly those targeting SASP regulation such as mTOR or IL-6 – may be effective in patients who have failed antiangiogenic therapy.

Disclosure of Potential Conflicts of Interest

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