RESEARCH HIGHLIGHT

Senolysis through thrombomodulation

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In a recent study published in *Cell Research*, Pan et al. show that thrombomodulin (THBD), a cell surface glycoprotein activator of the thrombin-mediated MAPK pathway, is a key determinant of senescent cell fate. Using the FDA-approved THBD pathway inhibitor, vorapaxar, or depletion of THBD signaling, they show that inhibition or depletion of the THBD pathway acts as a novel senolytic mechanism and restores homeostasis in liver fibrosis models.

In response to most cellular stresses, cells activate p53 to impose a p21-dependent cell cycle arrest to allow for repair or adaptation (Fig. 1). Cells that fail to recover typically die through apoptosis or enter a senescent state characterized by permanent arrest and a senescence-associated secretory phenotype (SASP) that induces pathology in neighboring cells and the tissue at large.[1,2] However, the molecular mechanisms that mediate cell survival in both the pre-senescent and the senescent states remain incompletely understood, which has limited the development of effective senotherapeutic strategies to eliminate pathology-causing senescent cells (SNCs). In a series of elegant experiments, Pan et al. demonstrate a critical role for thrombomodulin (THBD) in the irreversibility of cellular senescence and SNC viability through the stabilization of a protein complex in recycling endosomes, which prevents proteasome formation, suppresses caspase-3 cleavage, and sequesters factors that facilitate apoptosis. By using vorapaxar, the authors identify PAR1 as a novel senolytic target in the THBD pathway.

Pan et al. show that THBD is upregulated upon exposure of cultured cells to diverse senescence-inducing stressors and elevated in various aged tissues and organs.[3] Furthermore, they demonstrate that elevated THBD is necessary and sufficient to drive cells into a senescent state and critical for survival once cells have become senescent through suppression of caspase-3 cleavage. The key THBD target here is the G-protein coupled receptor PAR1, which upon THBD-mediated cleavage at Arg46 activates its cytoprotective and anti-proliferative signaling. PAR1 signaling is normally quenched through receptor complex internalization and its proteolytic and lysosomal degradation, but elevated THBD suppresses NEDD4L-mediated ubiquitination, thereby perpetuating PAR1 signaling and suppression of caspase-3 activation. The authors affirm the critical role of PAR1 signaling in establishing SNCs and sustaining their viability by using vorapaxar (Fig. 1), a PAR1 antagonist clinically used as an antiplatelet drug in the treatment or prevention of myocardial infarction and stroke.[4] In a final set of experiments, Pan et al. employ animal models of liver fibrosis characterized by extensive cellular senescence to explore the potential clinical use of vorapaxar as a senolytic. Indeed, they find that these models accumulate fewer SNCs and develop less fibrotic tissue while on vorapaxar.

These exciting data expand the repertoire of mechanisms that both steer stressed cells away from apoptosis in favor of senescence and control cell survival once senescence has been established. At the earliest stages in the procession towards cellular senescence, once sensors detect for instance genotoxic, oxidative, or mitochondrial damage, an immediate-early cell stress response is initiated.[5] One of the first senescence-related proteins that gets activated is p53, which translocates to the nucleus where it induces the transcription of various pro-apoptotic genes and the cyclin-dependent kinase inhibitor p21, among many other genes (Fig. 1).[1] Through Rb hypophosphorylation, p21 not only promptly halts the cell cycle of stressed cells via transcriptional inhibition of E2F target genes but also activates a wide variety of genes, including nearly half the genes constituting the SASP, referred to as the p21-associated secretory phenotype (PASP).[6] One of the p21-dependent genes immediately induced upon cellular stress is RNASE4, a p53 binding partner that inhibits p53-mediated apoptosis in favor of senescence and promotes senescent survival (Fig. 1).[6,7] A second RNASE, RNASE5 (or Ang), has very similar properties but is not p21 controlled. Intriguingly, THBD has previously been identified as an immediate-early p21-controlled gene, and by virtue of its secretion, been classified as a PASP factor.[5] BCL-xL is a fourth prosurvival factor with a role in suppressing apoptosis both on the path to senescence and in the senescent state. This mitochondrial inhibitor of p53 is the target of UBX1325, a senolytic drug that has shown clinical efficacy in patients with diabetic macular degeneration (NCT04857996).[8] Despite the tremendous importance of this proof-of-principle clinical trial, UBX1325 is not suitable for age-related conditions that require systemic distribution of senolytic agents, a limitation that vorapaxar may help resolve.

The intriguing new findings by Pan et al. raise several interesting questions for future studies. While THBD is a PASP factor controlled by p21 through Rb hypophosphorylation, it in turn is shown to activate p21. This raises the possibility that a feed-forward loop exists that may have relevance for the propensity of induction of the permanent senescence pathway. An interesting question that arises regarding the activity of vorapraxar as a systemic senolytic relates to the kinetics of PAR1-mediated senescence stabilization. As a p53-dependent gene, p21 induces a temporal cell cycle arrest to allow stressed cells to recuperate and reengage in proliferation, and only if they are beyond repair, apoptosis or senescence mechanisms should

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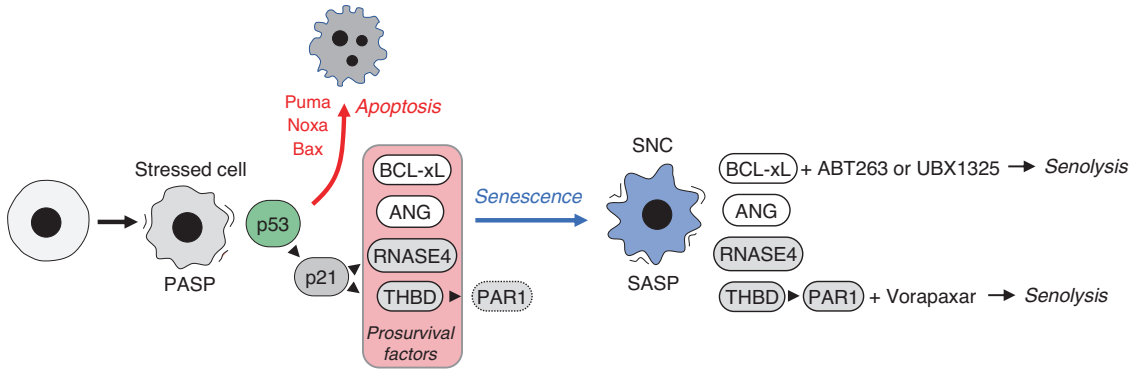

Fig. 1 THBD joins a subset of immediate-early stress response factors that promote the formation and survival of SNCs. Most cellular stresses activate p53, which in turn induces various pro-apoptotic target genes as well as p21, which dramatically alters the transcriptome through hypophosphorylation of the Rb transcription factor, resulting in rapid cell cycle arrest and concomitant generation of the PASP. The PASP includes RNASE4 and THBD, which together with two other immediate-early stress response proteins, ANG and BCL-xL, mount a prosurvival response that counteracts apoptosis and steer cells beyond repair to the alternative cell fate of cellular senescence. Once senescent, cells continue to depend on each of these immediate-early survival factors, as illustrated by the observation that their depletion causes SNC death. Small-molecule senolytics have been identified that target BCL-xL or THBD for inhibition.

proceed. If vorapaxar were to promote apoptosis at this early stage, its mechanism of action as a senolytic would target both pre-senescent and senescent cells for elimination. Furthermore, since in the liver fibrosis animal models, initiation of vorapraxor treatment coincided with the application of the various fibrosis-inducing stressors, it is not yet entirely clear whether vorapaxar merely prevents pathology or can reverse fibrosis in animals with established or ongoing liver pathology. As a final note, THBD exists not only on the cell surface and in endosomes, but also in secreted form.[9] Previous work has shown beneficial effects of treatment with soluble human THBD in patients with disseminated intravascular coagulation because of hematologic malignancy, as well as in animal models of sepsis with decreased inflammatory markers.[10,11] Interestingly, many of the markers of inflammation shown to be reduced by recombinant THBD overlap with inflammatory markers of the SASP. It would therefore be interesting to further explore whether secreted THBD also contributes to senescence induction in a manner similar to endosomal THBD, as the authors have shown. Taken together, Pan et al. demonstrate a novel mechanism of irreversibility in senescence and pioneer a route toward a novel FDA-approved senolytic. Perhaps a clue of its potential as a human senolytic could be obtained from the analysis of patients who have been chronically administered with the drug for the mitigation of cardiovascular disease.

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ADDITIONAL INFORMATION

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