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REVIEW

Functional Role of Cellular Senescence in Biliary Injury

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Cellular senescence is a state of irreversible cell cycle arrest that has been involved in many gastrointestinal diseases, including human cholestatic liver disorders. Senescence may play a role in biliary atresia, primary sclerosing cholangitis, cellular rejection, and primary biliary cirrhosis, four liver diseases affecting cholangiocytes and the biliary system. In this review, we examine proposed mechanisms of senescence-related biliary diseases, including hypotheses associated with the senescence-associated phenotype, induction of senescence in nearby cells, and the depletion of stem cell subpopulations. Current evidence for the molecular mechanisms of senescence in the previously mentioned diseases is discussed in detail, with attention to recent advances on the role of pathways associated with senescence-associated phenotype, stress-induced senescence, telomere dysfunction, and autophagy. *(Am J Pathol 2015, 185: 602–609; http://dx.doi.org/10.1016/j.ajpath.2014.10.027)*

Cellular senescence is a state of irreversible growth arrest in the G_1 phase of the cell cycle.[1,2] Telomere shortening, double-stranded DNA damage, inflammation, and other forms of cell stress all function as stimuli for cell senescence. Although traditionally cellular senescence has been viewed as a protective response to cellular injury or disruption, the study of the senescence-associated secretory phenotype (SASP) has become increasingly implicated in the pathogenesis of hepatobiliary disease. Specifically, senescence has been hypothesized to play a prominent role in cholangiopathies, including primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), cellular rejection (CR), and biliary atresia (BA). Over time, the SASP may provide proinflammatory or other adverse stimulus to hepatobiliary stem/progenitor cells, leading to the observed disease phenotypes.[3–5] This review summarizes current evidence for the molecular mechanisms of cellular senescence in the pathogenesis of select biliary diseases.

Cellular Senescence

First observed in 1965, human fibroblasts were noted to have a limited ability to replicate in culture.[6] Senescent cells are metabolically active cells that often remain *in situ* but no longer maintain proliferative activity. This development is associated with morphological changes causing the cells to appear large and flat with characteristic nuclear changes, including vacuolation.[7–10] Two main tumor-suppressor pathways, p53 and $p16^{INK4a}$/pRB, have been shown to regulate senescence responses.[11] The expression of associated cell cycle inhibitors, including $p15^{INK4B}$, $p16^{INK4a}$, $p21^{WAF1/Cip1}$, p53, and increased senescence-associated β-galactosidase (SA-β-GAL) activity, is commonly observed. In addition, the production of senescence-associated heterochromatin foci and DNA damage response proteins have all been used to aid in the identification

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Table 1 Summary of Effects of the Consequences on Various Cell Types

Cell type	Consequences of senescence*
Hepatocytes	Unclear, but senescence correlated with fibrosis stage[13]
Cholangiocytes	Unclear, but senescence correlated with Banff grade in acute rejection[14–16]
Hepatic stellate cells	Ameliorated fibrosis during acute liver damage via secretion of metalloproteinases[17]
Retinal pigment epithelial cells	Disruption of division and migration and atrophy of retina in adult macular degeneration[18]
Fibroblasts	Disrupted tissue structure locally via secretion of VEGF[1]
Melanocytes	Reinforcing senescence via secretion of IL-6, IL-8, and PAI-1[19]

*Senescence causes different effects in different tissues and cell types.
PAI, plasminogen activator inhibitor; VEGF, vascular endothelial growth factor.

of senescent cells in association with the absence of replicative markers (Ki-67 or thymidine analogue bromodeoxyuridine *in vitro*).[12] Overall, senescence has been shown to cause different effects in different tissues and cell types (Table 1). Correlating expression of these markers with disease states has demonstrated the widespread prevalence of the senescent states in hepatobiliary diseases.

Replicative Senescence

Multiple effectors have been identified leading to the development of senescence. Classically, replicative senescence develops from telomere erosion associated with the accumulation of replication cycles (aging) in the absence of corrective telomerase (Figure 1). The process of telomere erosion elicits a DNA damage response, which acts to induce the expression of γ-H2Ax (a phosphorylated histone variant of H2Ax) and the DNA damage response proteins p53-binding protein 1, nibrin, and mediator of DNA damage checkpoint protein 1. DNA damage kinases ataxia telangiectasia mutated and ataxia telangiectasia and Rad3-related protein are subsequently activated and, in turn, activate checkpoint kinases 1 and 2.[20,21] The resultant signal cascade leads to differential expression of p53 isoforms, and has been linked directly to senescent phenotypes, although the process, including breakpoints between apoptosis and senescence, remains incompletely understood, with some role of NF-κB in its regulation.[22,23] In cholestatic liver disease, the role of age-related telomere erosion does not seem prominent. In a study of telomeres using quantitative fluorescent *in situ* hybridization (Q-FISH) and confirmed by Southern blot analysis from 73 normal liver samples, cholangiocytes and hepatocytes were shown to maintain their telomere lengths independent of age with only Kupffer and stellate cells, demonstrating age-related attrition.[24]

Premature Senescence

Numerous other mechanisms independent of replicative senescence have been identified as pathways of so-called premature senescence, including chromatin instability, DNA damage, dysfunctional telomeres, oncogenic mutations, overexpression of cell cycle inhibitors, and stress signals

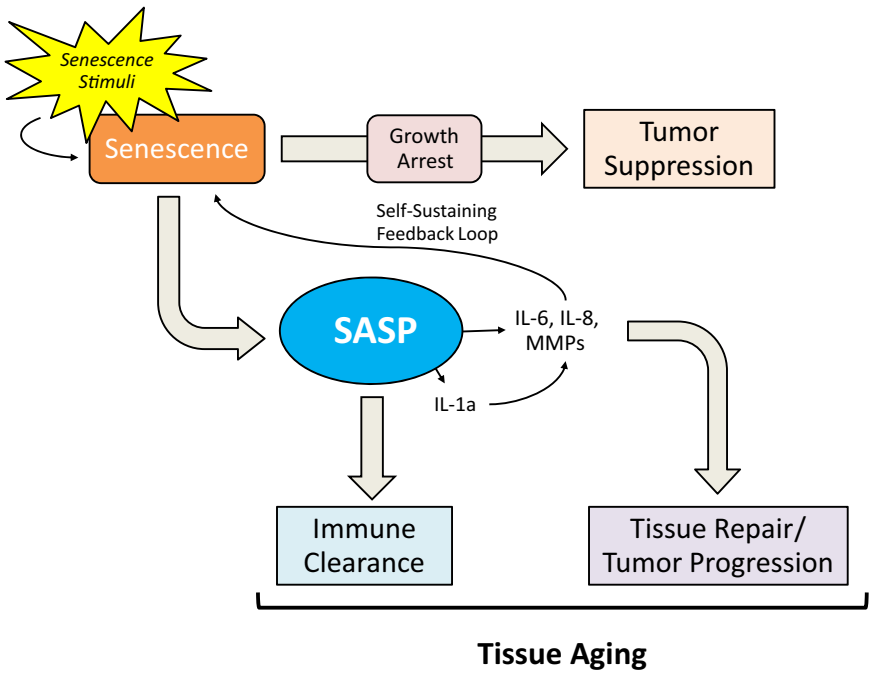

Figure 1 The senescence-associated secretory phenotype (SASP) during aging, cancer development, and progression. Cellular senescence has dual effects on human health during the progression of aging and various human disorders. The beneficial effects include tumor suppression and tissue repair after injury; the deleterious effects include tumor and aging promotion that involve several key SASP molecules, including IL-6, IL-8, matrix metalloproteinases (MMPs), and specific miRNAs.