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Practice points

- Cellular senescence is a stress-induced reversible cell-cycle arrest program.
- Senescent cells secrete cytokines and chemokines that shape the function and composition of their immune microenvironment.
- Collectively, the secreted cytokines are termed 'senescence secretome' or 'senescence-associated secretory phenotype' (SASP).
- Cellular senescence in hepatic stellate cells can limit liver fibrosis by decreasing extracellular matrix deposition, enhancing matrix degradation and inducing their own clearance through natural killer cells.
- Cellular senescence in hepatocytes prevents liver cancer development by prompting their own clearance through a concerted effort of CD4 T cells and macrophages.
- Liver tumors can skew senescence-recruited myeloid cells toward immunosuppression; thereby promoting tumor growth.
- In obesity, gut bacterial metabolites induce senescence in hepatic stellate cells that restrain antitumor CD8 T-cell responses by inhibiting CD103⁺ dendritic cells through a prostaglandin E2-dependent mechanism.
- Given the implications of SASP effector functions in tumor development and progression, altering the SASP might provide new therapeutic avenues for the treatment of liver cancer.

Cellular senescence is a stress-induced cell-cycle arrest program that prevents malignant transformation of senescent cells following oncogenic pathway activation and DNA damage. Senescent cells are metabolically active and secrete cytokines and chemokines that shape the function and composition of their microenvironment. These cytokines can recruit immune cells such as lymphocytes and myeloid cells that depending on the context can either promote or inhibit liver tumor development and progression. Accordingly, pharmacologically targeting of secreted cytokines or reprogramming the expression of these cytokines in senescent cells represents a promising approach to skew senescence-associated immune responses toward cancer cell killing.

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Cellular senescence: background

Cellular senescence is a stress-induced reversible cell-cycle arrest program, which induces proliferative arrest in cells at risk of malignant transformation through stress response pathways by activation of molecules such as p53 and p21 [1–4]. Recently, cellular senescence was also found to be important for physiological processes not associated with cellular stress, such as embryonic development. Among the factors that induce the stress-associated form of cellular senescence are activation of oncogenic pathways, inactivation of tumor suppressors, DNA damage and telomere attrition. Therefore, cellular senescence is widely considered to be an antitumor mechanism [5–7].

KEYWORDS

- hepatocellular carcinoma
- immune cells • liver • SASP
- senescence • tumor

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In addition to this cell autonomous mechanism of cancer prevention and in contrast to quiescent cells, senescent cells are metabolically active and secrete different cytokines and chemokines such as IL-1, IL-6 and CCL2 [8–10]. Collectively, the secreted cytokines are termed senescence secretome or senescence-associated secretory phenotype (SASP). The SASP affects the function of neighboring parenchymal cells, stromal cells and immune cells. For example, SASP factors can trigger senescence in an autocrine and paracrine fashion and thereby enhance the antitumorigenic effect by either reinforcing the senescence program or increasing the threshold of induction of cell cycle progression, respectively [8,10]. Ultimately, SASP factors promote stabilization of the senescence program and prevent senescence escape.

Importantly, the senescence-induced cell cycle arrest is reversible and thus, tumor development can occur from previously senescent cells once they have escaped the senescence program. Moreover, depending on the context, the SASP can also have protumorigenic effects on neighboring cells by inducing proliferation via cytokines and growth factors [11,12]. SASP factors are capable of inducing epithelial–mesenchymal transition and invasiveness primarily by paracrine activation of IL-6 and IL-8 signaling in premalignant cells [13]. Another example of detrimental consequences of cellular senescence induction was shown for breast cancer. Mammary tumors incapable of senescence induction because of inactivating p53 mutations exhibited a superior clinical response to chemotherapy compared with tumors that senesced upon chemotherapy treatment. Mechanistically, chemotherapy-induced senescence caused secretion of mitogenic cytokines by tumor cells to accelerate malignant transformation of neighboring cells. In tumor cells incapable of senescence induction, chemotherapy treatment resulted in failure to arrest cell cycle progression, abnormal mitoses and cell death [14]. In summary, different oncogenic stimuli can initiate the cellular senescence program, resulting in proliferation arrest and metabolic changes, including secretion of cytokines, that is, the SASP.

Cellular senescence in the liver

The vast majority of hepatocellular carcinomas arise in chronically inflamed livers. Chronic liver inflammation causes repetitive waves of hepatocyte death and compensatory regeneration of hepatocytes. This process in turn can

lead to aberrant activation of oncogenes, which ultimately induce the senescence program. Studies addressing the consequences of cellular senescence induction in hepatocytes as well as hepatic stellate cells revealed critical functions in protecting from or aiding in hepatocellular tumor development. Most studies, however, have linked cellular senescence in the liver predominantly to inhibition of hepatocellular carcinoma (HCC) development and progression [15–18]. For example, hepatocytes that acquire the SASP upon activation of the NRas signaling cascade recruit CD4 T cells and macrophages to clear the senescent hepatocytes in a coordinated effort. Thereby, the SASP prevents NRas-induced hepatocarcinogenesis, a process termed ‘senescence surveillance’ [16]. Consequently, in the absence of macrophages or CD4 T cells, NRas-expressing hepatocytes persist, that is, are not cleared, ultimately escape the senescence program and initiate liver tumor development. Further evidence for the postulation of cellular senescence as a cancer protective mechanism stems from the finding that oncogenic mutations in hepatocytes, for example, inactivating p53 mutations, that preclude induction of the senescence program, can cause aggressive HCC development [19]. Moreover, restoration of p53 in liver tumors can reinstate the senescence program, triggers SASP factor release and elicits a natural killer (NK) cell-dependent immune response resulting in killing of these senescent liver tumor cells [15,18]. Since hepatitis B and C virus infections are the most common etiologies of hepatocellular carcinoma development, these viruses are required to blunt senescence-induced cell cycle arrest in order to cause tumorigenesis. Indeed, hepatitis B virus X protein and hepatitis C virus core protein interfere with the senescence response through reduction of Notch1 cleavage and downregulation of p16, respectively [20,21].

Liver fibrosis constitutes a precursor of liver tumor development and hepatic stellate cells are the main contributor to matrix production and fibrosis development. Induction of cellular senescence in hepatic stellate cells, however, can limit liver fibrosis by decreasing extracellular matrix deposition and enhancing matrix degradation [22]. Furthermore, these senescent activated hepatic stellate cells are cleared by natural killer cells, which presumably are recruited through a SASP-dependent mechanism. One mechanism of senescence induction in hepatic stellate cells

is IL-22-receptor signaling that activates p53 in a STAT3-dependent manner [23]. Accordingly, in patients with hepatitis B, an inverse correlation of hepatic IL-22 expression and fibrosis stage has been reported [24]. Nevertheless, activation of cellular senescence in hepatic stellate cells can also promote hepatocellular carcinoma development. Obesity, a newly recognized risk factor for hepatocellular carcinoma development, can cause alterations in the composition of the gut microbiota. Obesity-associated gram positive bacterial metabolites, that is, deoxycholic acid and lipoteichoic acid, trigger senescence induction and SASP factor expression in hepatic stellate cells. These proinflammatory SASP factors accelerate hepatocellular carcinogenesis [25,26]. Taken together, cellular senescence in the liver is primarily associated with inhibition of tumor development and growth. Yet, in certain conditions, such as obesity, proinflammatory factors associated with the SASP can promote tumorigenesis.

The effect of the SASP on immune responses

Cytokines and chemokines associated with the SASP can regulate hepatic immune responses. As noted above, senescence-recruited macrophages clear senescent hepatocytes to protect from liver tumor development [14]. The SASP factor responsible for macrophage recruitment and accumulation was found to be CCL2 (Figure 1A) [27]. In this setting, senescent hepatocytes are removed before tumor formation can occur. Nevertheless, oncogenic mutations, such as inactivating p53 mutations, can result in abrogation of the senescence program and subsequent initiation of hepatocarcinogenesis [17]. Therefore, when tumor development does occur, some of the senescent hepatocytes in chronically inflamed livers sustain a senescence-abrogating mutation due to the ongoing inflammatory stimuli that trigger cell cycle progression and malignant transformation. The ensuing tumor cells are surrounded by persisting senescent hepatocytes. In this setting, however, senescent hepatocyte-secreted CCL2 recruits immature myeloid cells that do not mature into macrophages because of tumor cell secreted factors [27]. These immature myeloid cells in turn inhibit antitumor NK cell responses (Figure 1B). NK cell inhibition then allows for accelerated tumor growth. In summary, hepatocellular senescence in the absence of neighboring full-blown tumor cells functions as a protective anticancer mechanism. In the

presence of tumor cells, nontumorous senescent hepatocytes enhance tumor-associated immune suppression by expanding immunosuppressive immature myeloid cells.

In obesity, gut bacterial metabolites induce cellular senescence in hepatic stellate cells [25]. Senescent hepatic stellate cell SASP factors can restrain antitumor CD8 T-cell responses through a COX2-dependent production of PGE2 [26]. Specifically, PGE2, produced by senescent stellate cells, engages PTGER4 on immune cells to inhibit CD103⁺ dendritic cells and reduce activation of CD8 T cells (Figure 1C). Collectively, these data demonstrate that cellular senescence, through SASP factor release, regulates critical tumor-associated immune responses in the liver.

Targeting the SASP to skew immune responses toward cancer cell killing

One approach to target the SASP would be to interfere with secreted cytokines and chemokines that promote tumor growth. In patients with liver cancer, the SASP factor CCL2 was identified to be critical for the expansion of immunosuppressive immature myeloid cells, which inhibited antitumor NK cell responses and worsened survival of patients with HCC [27]. Thus, blockade of the CCL2–CCR2 axis seems to be an interesting target to alleviate senescence-induced tumor growth promotion. An alternative approach to target the SASP would be to alter the SASP altogether.

Studies investigating pharmacological reprogramming of the SASP have not yet been conducted in hepatocellular carcinomas. However, such a study was carried out in a preclinical model of prostate cancer [28]. Senescent prostate tumor cells exhibit a SASP that creates an immunosuppressive tumor microenvironment consisting of myeloid-derived suppressor cells that restrain antitumor CD8 T cells. Expression of SASP factors that cause accumulation of myeloid-derived suppressor cells was driven by JAK2/STAT3-pathway signaling. Hence, efficacy of chemotherapy was enhanced when it was combined with JAK2/STAT3 inhibitors that resulted in an SASP driven by NF-κB signaling. This treatment effectively reprogrammed the SASP to allow for infiltration into the tumor microenvironment by antitumor immune cells, such as CD8 T cells and NK cells. Given the implications of SASP effector functions in tumor development and progression in hepatocellular carcinoma, altering the SASP might provide new therapeutic avenues for this

Figure 1. Regulation of hepatic immune responses through cellular senescence. (A) Senescent hepatocytes secrete CCL2 to recruit iMC, which mature into macrophages to clear senescent hepatocytes. (B) HCC impede the maturation of iMC, which inhibit NK cell-mediated tumor clearance. Senescent hepatocyte-secreted CCL2 enhances the accumulation of immunosuppressive iMC. (C) Obesity-associated gram positive bacterial metabolites, that is, DCA and LTA, trigger senescence induction and COX2-dependent production of PGE₂, which inhibits antitumor function of CD8 T cells.

DCA: Deoxycholic acid; HCC: Hepatocellular carcinoma; iMC: immature myeloid cell; LTA: Lipoteichoic acid; NK: Natural killer.

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disease. Nevertheless, it is critical to consider the context-specific and cell-type-specific nature of the functional outcome of cellular senescence in the liver. As explained above, the SASP can mount beneficial NK cell-driven immune responses by limiting fibrosis development and killing senescent liver tumor cells. Targeting the SASP in liver cancer therefore needs to ensure that tumor-promoting effects of the SASP are skewed toward inhibition of cancer growth while at the same time sparing the beneficial aspects of the SASP in liver cancer.

Conclusion & future perspective

Recent research on cellular senescence in the liver has revealed critical functions of this cell-cycle arrest program on hepatic immune responses and their consequences for liver tumor development and progression. Only now have we begun

to unravel the interplay of cytokines secreted by either senescent cells or tumor cells and their combined contribution to the tumor immune environment. Accordingly, cellular senescence might be an underestimated component in shaping the tumor microenvironment compared with the tumor alone, specifically in the context of distinct underlying liver diseases and/or comorbidities such as liver fibrosis and obesity. Future studies will advance our understanding of senescence-associated immune responses in liver cancer. Gained insights will then be translated into either pharmacological targeting of individual SASP factors or into reprogramming the SASP altogether.

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References

Papers of special note have been highlighted as:

• of interest; •• of considerable interest

- 1 Braig M, Lee S, Loddenkemper C *et al.* Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature* 436, 660–665 (2005).
- 2 Chen Z, Trotman LC, Shaffer D *et al.* Crucial role of p53-dependent cellular senescence in suppression of PTEN-deficient tumorigenesis. *Nature* 436, 725–730 (2005).
- 3 Michaloglou C, Vredeveld LC, Soengas MS *et al.* BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 436, 720–724 (2005).
- 4 Bartkova J, Rezaei N, Lontos M *et al.* Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature* 444(7119), 633–637 (2006).
- 5 Acosta JC, Gil J. Senescence: a new weapon for cancer therapy. *Trends Cell. Biol.* 22(4), 211–219 (2012).
- 6 Narita M, Lowe SW. Senescence comes of age. *Nat. Med.* 11(9), 920–922 (2005).
- 7 Nardella C, Clohessy JG, Alimonti A, Pandolfi PP. Pro-senescence therapy for cancer treatment. *Nat. Rev. Cancer* 11(7), 503–511 (2011).
- 8 Acosta JC, Banito A, Wuestefeld T *et al.* A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat. Cell Biol.* 15(8), 978–990 (2013).
- 9 Coppe JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu. Rev. Pathol.* 5, 99–118 (2010).
- 10 Kuilman T, Michaloglou C, Vredeveld LC *et al.* Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* 133(6), 1019–1031 (2008).
- 11 Jackson JG, Pant V, Li Q *et al.* p53-mediated senescence impairs the apoptotic response to chemotherapy and clinical outcome in breast cancer. *Cancer Cell* 21(6), 793–806 (2012).
- 12 Perez-Mancera PA, Young AR, Narita M. Inside and out: the activities of senescence in cancer. *Nat. Rev. Cancer* 14(8), 547–558 (2014).
- 13 Coppé JP, Patil CK, Rodier F *et al.* Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* 6(12), 2853–2868 (2008).
- 14 Jackson JG, Pant V, Li Q *et al.* p53-mediated senescence impairs the apoptotic response to chemotherapy and clinical outcome in breast cancer. *Cancer Cell* 21(6), 793–806 (2012).
- 15 Iannello A, Thompson TW, Ardolino M, Lowe SW, Rauler DH. p53-dependent chemokine production by senescent tumor cells supports NKG2D-dependent tumor elimination by natural killer cells. *J. Exp. Med.* 210(10), 2057–2069 (2013).
- 16 Kang TW, Yevsa T, Woller N *et al.* Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature* 479(7374), 547–551 (2011).
- Described the senescence-induced and immune-cell-mediated clearance of senescent hepatocytes at risk of malignant transformation.
- 17 Lujambio A, Akkari L, Simon J *et al.* Non-cell-autonomous tumor suppression by p53. *Cell* 153(2), 449–460 (2013).
- 18 Xue W, Zender L, Miething C *et al.* Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 445(7128), 656–660 (2007).
- 19 Mudbhary R, Hoshida Y, Chernyavskaya Y *et al.* UHRF1 overexpression drives DNA hypomethylation and hepatocellular carcinoma. *Cancer Cell* 25(20), 196–209 (2014).
- Establishes the sequence of oncogene activation, senescence induction, senescence escape and hepatocarcinogenesis.
- 20 Xu J, Yun X, Jiang J *et al.* Hepatitis B virus X protein blunts senescence-like growth arrest of human hepatocellular carcinoma by reducing Notch1 cleavage. *Hepatology* 52(1), 142–154 (2010).
- 21 Lim JS, Park SH, Jang KL. Hepatitis C virus core protein overcomes stress-induced premature senescence by down-regulating p16 expression via DNA methylation. *Cancer Lett.* 321(2), 154–161 (2012).
- 22 Krizhanovsky V, Yon M, Dickins RA *et al.* Senescence of activated stellate cells limits liver fibrosis. *Cell* 134(4), 657–667 (2008).
- Revealed a mechanism of liver fibrosis reduction through activation of cellular senescence in activated hepatic stellate cells.
- 23 Kong X, Feng D, Wang H *et al.* Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis in mice. *Hepatology* 56(3), 1150–1159 (2012).
- 24 Xiang X, Gui H, King NJ *et al.* IL-22 and non-ELR-CXC chemokine expression in chronic hepatitis B virus-infected liver. *Immunol. Cell Biol.* 90(6), 611–619 (2011).
- 25 Yoshimoto S, Loo TM, Atarashi K *et al.* Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 499(7456), 97–101 (2013).
- 26 Loo TM, Kamachi F, Watanabe Y *et al.* Gut microbiota promotes obesity-associated liver cancer through PGE2-mediated suppression of antitumor immunity. *Cancer Discov.* 7(5), 522–538 (2017).
- Demonstrates the interplay between obesity, gut microbiota, senescence of hepatic stellate cells and inhibition of antitumor immune responses.
- 27 Eggert T, Wolter K, Ji J *et al.* Distinct functions of senescence-associated immune responses in liver tumor surveillance and tumor progression. *Cancer Cell* 30(4), 533–547 (2016).
- Establishes a link between nontumorous senescent liver tissue and tumor-associated immunosuppression and tumor growth promotion.
- 28 Toso A, Revandkar A, Di Mitri D *et al.* Enhancing chemotherapy efficacy in pten-deficient prostate tumors by activating the senescence-associated antitumor immunity. *Cell Rep.* 9, 1–15 (2014).
- Lays the conceptual groundwork for reprogramming the senescence-associated secretory phenotype to skew senescence-associated immune responses toward cancer cell killing.