Molecules and Cells

Minireview

Transcriptional Heterogeneity of Cellular Senescence in Cancer

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Cellular senescence plays a paradoxical role in tumorigenesis through the expression of diverse senescence-associated (SA) secretory phenotypes (SASPs). The heterogeneity of SA gene expression in cancer cells not only promotes cancer stemness but also protects these cells from chemotherapy. Despite the potential correlation between cancer and SA biomarkers, many transcriptional changes across distinct cell populations remain largely unknown. During the past decade, single-cell RNA sequencing (scRNA-seq) technologies have emerged as powerful experimental and analytical tools to dissect such diverse senescence-derived transcriptional changes. Here, we review the recent sequencing efforts that successfully characterized scRNA-seq data obtained from diverse cancer cells and elucidated the role of senescent cells in tumor malignancy. We further highlight the functional implications of SA genes expressed specifically in cancer and stromal cell populations in the tumor microenvironment. Translational research leveraging scRNA-seq profiling of SA genes will facilitate the identification of novel expression patterns underlying cancer susceptibility, providing new therapeutic opportunities in the era of precision medicine.

Keywords: cancer, cellular heterogeneity, senescence, single-cell RNA sequencing

INTRODUCTION

The development of cancer is suppressed by many tumor suppressor genes. Many of these genes permanently arrest the growth of cells at risk of neoplastic transformation via a process known as cellular senescence (Ben-Porath and Weinberg, 2004; Campisi and d'Adda di Fagagna, 2007; Dimri, 2005). Senescence is also characterized by a senescence-associated (SA) secretory phenotype (SASP) in which cells produce and secrete inflammatory cytokines, such as interleukin (IL)-6 and IL-8, chemokines, matrix metalloproteinases (MMPs), growth factors, and angiogenic factors (Kim and Park, 2019; Panda et al., 2017). These phenotypic factors combine many aspects of cell physiology and decide the fate of the cell, i.e., survival, death, proliferation, or stagnant growth, demonstrating the context-dependent broad spectrum of SASP (Cuollo et al., 2020; Sikora et al., 2021). Certain changes in SA transcripts observed in aging organisms are associated with cancer (Aramillo Irizar et al., 2018; Campisi, 2005; Kim and Park, 2019). However, it is not fully understood how these changes in gene expression contribute to cancer-related pathology.

It is estimated that demographic changes will increase the cancer burden by 47% over the next 20 years, significantly increasing cancer mortality (Bray et al., 2021; Sung et al., 2021). Researchers have set the goal of analyzing the characteristics of carcinogenic gene expression to identify changes at various intervals after the induction of cellular senescence.

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The identification of novel transcriptome signatures to detect all types of senescent cells or to differentiate between different senescence stages is an attractive strategy for deciphering various biological roles of senescent cells and developing specific drug targets. These characteristics include different gene expression patterns, deregulated cell–cell communication, aging, stem cell depletion, and epigenetic abnormalities that can lead to genomic instability (Lopez-Otin et al., 2013; Saul and Kosinsky, 2021).

Next-generation sequencing (NGS) approaches have greatly contributed to deciphering changes in gene expression signatures across different aging species and tissues at the transcriptome level (Schaum et al., 2020; Tabula Muris Consortium, 2020). Single-cell RNA sequencing (scRNA-seq) is a novel technique that enhances our understanding of complex intratumoral heterogeneity and addresses the question of whether distinct cell subpopulations exhibit dysregulation of cancer-associated genes (Lim et al., 2019a; 2019b; 2019c). This technique facilitates the investigation of cancer initiation and progression driven by temporal and spatial changes in gene transcription underlying aging processes, including chronic inflammation, immune proliferation, and senescence cytochemistry (Ou et al., 2021; Uyar et al., 2020). Novel subtypes of cells and their cell–cell communication mediated by ligand–receptor interactions have been characterized through single-cell approaches, revealing diverse effects of inflammation and SASP on different cell populations in cancer (Davalos et al., 2010; Freund et al., 2010; Uyar et al., 2020).

There is currently a need for an interdisciplinary approach leveraging single-cell data to develop the molecular clock, a biomarker signature of SASP predicting cancer or aging. In this review, we overview the paradoxical role of SASP in tumor progression and highlight the value of single-cell analysis in cancer research progress. Here, we present the expression landscape of cancer-causing SASP gene signatures in different cell types and compare scRNA-seq–derived findings from recently published studies on cancer, age-related chronic inflammation, and aging (i.e., cellular senescence and immune senescence).

PARADOXICAL ROLE OF SASP IN MALIGNANCY

Many DNA-damaging cellular stresses, including oncogene activation and DNA-damaging chemotherapy, can lead to cellular senescence (Kim and Park, 2019; van Deursen, 2014). Increasing molecular evidence indicates that the p53 and p16/Rb pathways are induced by exposure to chronic stress (i.e., DNA damage, oncogene expression, etc.), leading to cell cycle exit/arrest of stressed cells (Gorgoulis et al., 2019; Herranz and Gil, 2018). Upon the initiation of senescence, senescent cells progressively remodel their chromatin and start to sequentially implement other aspects of the senescence program, including SASP secretion, to enter into the next step called “full senescence” (Herranz and Gil, 2018). If these senescent cells persist for extended periods of time, they continue to the last step called “late senescence”, which can involve adaptation and diversification of the senescent phenotype (Herranz and Gil, 2018).

Although senescent cell cycle arrest is regulated by the p53 and p16/Rb tumor suppressor pathways, the SASP is controlled by enhancer remodeling and the activation of multiple transcription factors, such as the NF-κB, C/EBPβ, GATA4, mTOR, and p38MAPK signaling pathways (Gorgoulis et al., 2019; Herranz and Gil, 2018). NF-κB and C/EBPβ are activated in senescent cells and regulate SASP components by controlling transcription of key regulators of the inflammatory SASP, such as IL-8 and IL-6 (Birch and Gil, 2020; Herranz and Gil, 2018). The mTOR pathway is also an important node in SASP regulation by mediating IL-1α and MAPK-activated protein kinase 2 (MAPKAPK2) to control SASP (Birch and Gil, 2020; Faget et al., 2019; Herranz and Gil, 2018).

SASPs contain various components: interleukins; chemokines; other inflammatory molecules, such as TGF-β (transforming growth factor-β) and MIF (macrophage migration inhibitory factor); growth factors; MMPs; and insoluble factors, such as laminin and collagens (Coppe et al., 2010; Gorgoulis et al., 2019). Importantly, the specific composition and functions of the SASP vary depending on the cell type and the surrounding environment (Faget et al., 2019; Herranz and Gil, 2018). As shown in Fig. 1, SASPs can exhibit both tumor-promoting and tumor-suppressing roles with their diverse components and the surrounding tumor microenvironment (TME).

Tumor-promoting role of SASPs

The secretion of SASP leads to pleiotropic effects, including pathologically increased proliferation of precancerous and malignant cells (Coppe et al., 2010; Lecot et al., 2016). SASPs are also increasingly recognized as the driving force behind low-level chronic inflammation that causes or worsens many age-related diseases, such as cancer (Ferrucci and Fabbri, 2018; Furman et al., 2019). Additionally, SASPs can increase epithelial-mesenchymal transition (EMT) initiation, cancer cell stemness, invasion and metastasis, angiogenesis, and fibroblast activation, all of which promote tumors (Chambers et al., 2021). Studies have found that SASP factors stimulate epithelial cell invasion and EMT in tumor cells, enabling tumor cell migration and metastasis (Coppe et al., 2008; Laberge et al., 2012). Conditioned medium from senescent foreskin fibroblasts induced EMT in a breast cancer cell line through secretion of the proinflammatory cytokines IL-6 and IL-8, favoring tumorigenic processes (Ortiz-Montero et al., 2017). Increasing evidence suggests that SASP factors derived from senescent fibroblasts promote angiogenesis by stimulating endothelial cell infiltration *in vivo* (Faget et al., 2019). For example, coinjection of weakly malignant EpH4 epithelial cells with senescent fibroblasts increased the tumor vasculature through the production of vascular endothelial growth factor (VEGF) compared to controls (Coppe et al., 2006). Another SASP factor, connective tissue growth factor (CTGF), enhances angiogenesis and prostate cancer tumorigenesis *in vitro* and in an *in vivo* rat model (Ungvari et al., 2017; Yang et al., 2005). Of the other SASP factors, MMPs produced by senescent cells were found to promote metastasis through extracellular matrix (ECM) remodeling (Egeblad and Werb, 2002; Tsai et al., 2005), suggesting the therapeutic potential of MMP inhibitors in cancer therapy. In particular, dermal fibroblasts undergoing replication senescence secrete MMP1

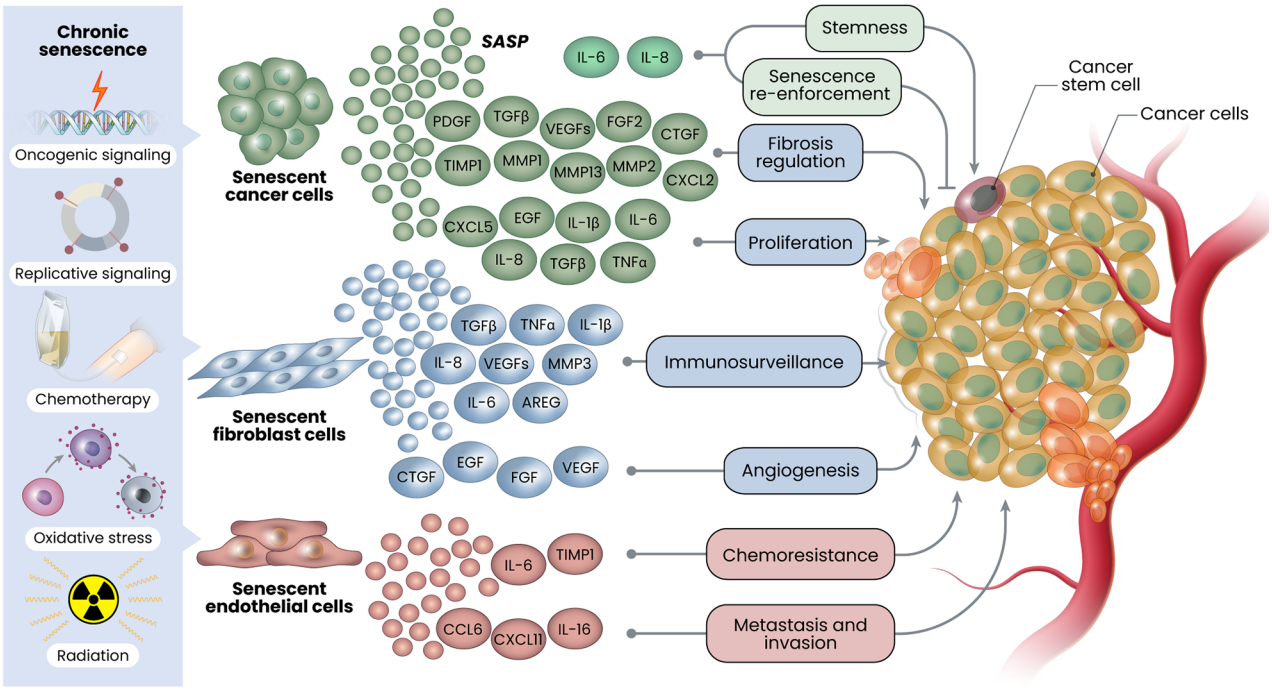

Fig. 1. Role of senescence-associated secretory phenotype (SASP) factors in tumor initiation and progression. Chronic senescence leads to DNA damage that elicits a DNA damage response *via* key effector pathways to execute senescence and SASP, promoting tumorigenesis (Campisi, 2011; Kim and Park, 2019; Lee and Schmitt, 2019). Various types of senescent cells present in the tumor microenvironment produce SASP factors, which are involved not only in auto and paracrine-mediated cell cycle arrest but also in tumor progression and chemoresistance (Bochenek et al., 2016; Hansel et al., 2020; Hassona et al., 2014; Liu and Cao, 2016). IL, interleukin; PDGF, platelet-derived growth factor; TGFβ, transforming growth factor beta; VEGFs, vascular endothelial growth factors; FGF, fibroblast growth factor; CTGF, connective tissue growth factor; TIMP, tissue inhibitors of metalloproteinases; MMP, matrix metalloproteinase; CXCL, chemokine (C-X-C motif) ligand; EGF, epidermal growth factor; TNF, tumor necrosis factor; AREG, amphiregulin; CCL, chemokine (C-C motif) ligand.

and MMP2 to induce activation of the serine/threonine protein kinase PAR1 in tumorigenic keratinocytes and stimulate their invasive activity (Heuberger and Schuepbach, 2019; Pittayapruek et al., 2016). Stromal cell SASP factors may also induce the development of tumor stem cells and defend tumor cells from chemotherapy by sheltering them from the immune response (Faget et al., 2019). Long-term SASP signaling can impair immune clearance and promote epithelial-mesenchymal metastasis and tumorigenesis in adjacent cells (Davalos et al., 2010; Demaria et al., 2017; Freund et al., 2010). In addition, there is a growing indication that senescent cells can re-enter the cell cycle, promoting tumor stem formation and ultimately contributing to tumor recurrence (Kumari and Jat, 2021; Milanovic et al., 2018).

Tumor-suppressing role of SASPs

Conversely, senescent cells can stimulate the adaptive immune response to inhibit tumorigenesis via SASPs (Faget et al., 2019). The antitumor roles of SASPs include arrest of the cell cycle of malignant cells and an increase in immune surveillance, which results in clearance of preneoplastic or senescent cancer cells by immune cells (Chambers et al., 2021; Herranz and Gil, 2018). Oncogene-induced senescence (OIS) functions as a tumor suppressor response that induces cell cycle arrest in response to oncogenic signaling (Kang et al., 2011). The OIS in otherwise normal murine hepatocytes inhibited liver cancer development through secretion of chemokines and cytokines that induced CD4+ T-cell recruitment and sequential immune-mediated clearance (Kang et al., 2011). More recently, oncogenic RAS-induced activation of p21, which is a main downstream target of p53, triggered immune surveillance and protected against oncogenic growth in the liver cancer setting (Sturmlechner et al., 2021). Interestingly, the p21-activated secretory phenotype (PASP) is distinct from the SASP in terms of kinetics and composition. Although SASPs are secreted from cells after cell cycle arrest (Herranz and Gil, 2018), cell cycle arrest and PASP are concurrently established, at least in hepatocytes (Sturmlechner et al., 2021). Although the SASP has various components depending on the cell type, surrounding environment, and senescence-inducing stressors (Coppe et al., 2010; Gorgoulis et al., 2019; Sturmlechner et al., 2021), PASP is enriched in immune-modulatory factors, such as CXCL14 that recruit macrophages to stressed cells (Sturmlechner et al., 2021). However, overexpression of p16, another important initiator of cellular senescence, did not result in CXCL14 or immune

Table 1. Summary of recently published scRNA-seq studies on cancer and senescence

Sample origin	Condition	No. of cells sequenced	No. of cell cluster	sc/snRNA-seq technology	Remarks	Reference
Human tissue	Pancreatic ductal adenocarcinoma	57,530	10	10x	The heterogeneous malignant subtype was composed of several subpopulations. Suppressed T-cell activation was associated with clinical pathological features.	(Peng et al., 2019)
Mice tissue (liquid biopsy)	Lung cancer	8,213	5	10x	In total, 19 tumor-specific markers for rare circulating tumor cells were identified.	(Dong et al., 2020)
Human tissue	Gastric cancer	32,332	17	10x	A single-cell network of premalignant lesions and early gastric cancer was constructed and characterized.	(Zhang et al., 2019)
Human tissue	Gallbladder cancer	24,887	10	BD Rhapsody	Immunosuppressive microenvironment was characterized as exhausted T cells and APOE+ macrophages.	(Chen et al., 2021)
Mice tissue	Lung cancer	3,891	12	10x, Smart-seq2	Transcriptional heterogeneity was observed in tumor cells in which p53 was inactivated.	(Marjanovic et al., 2020)
Human tissue	Gastric cancer	200,000	21	10x	An increase in KLF2 expression was found in gastric epithelial cancer cells compared to controls.	(Kumar et al., 2022)
Human tissue	Liver cancer	7,947	17	MARS-seq	Endothelial and pericytes cells showed SLIT-ROBO signaling interaction with tumor cells.	(Massalha et al., 2020)
Human tissue	Breast cancer	19,000	8	10x	CAF (cancer associated fibroblasts) subclusters and TGF-β signaling contributed to immunotherapy resistance.	(Kieffer et al., 2020)
Human tissue	Breast cancer	45,000	17 T cells: 14 myeloid cells	Drop-seq	Regulatory T-cell subpopulations exhibited (1) coexpression of CTLA-4, TIGIT and GITR to prevent pro-inflammatory response, and (2) an expansion in immune phenotypic space in breast tumor cells compared to normal cells.	(Azizi et al., 2018)
Human tissue	Pancreatic neuroendocrine tumor	24,544	10	10x	Increased PCSK1 and SMOC1 expression levels were observed in tumors with metastatic potential compared to controls.	(Zhou et al., 2021)
Human tissue	Lung fibrosis	76,070	14	10x, Smart-seq2	Wnt ligands and *AXIN2*/*Axin2* expression was observed in human lungs with pulmonary fibrosis.	(Reyfman et al., 2019)
Mouse tissue	Fibroblast heterogeneity	6,158	16	10x, Smartseq2	(1) Epigenetic changes contributed to the observed heterogeneity in fibroblasts. (2) High COL12A1, FOXL1 and WIF1 expression were observed in fibroblasts, leading to pathological changes in ECM.	(Muhl et al., 2020)
Human skin	Aging/senescence	15,457	17	10x	Increased SFRP2 expression was observed compared to FMO1 in all aged-dermal fibroblasts.	(Sole-Boldo et al., 2020)
Mice tissue	Aging/senescence	~50,000	38	10x	The senescence signaling pathway was activated in epithelial cell clusters.	(Ximerakis et al., 2019)
Mice tissue	Aging/senescence	~350,000	13	10x, Smart-seq2	An increase in P16 expression was found in old mice compared to controls.	(Tabula Muris Consortium, 2020)
Mouse tissue	Aging/senescence	4,233	13	10x	A substantial number of senescent endothelial cells was observed in the mouse cerebral microcirculation.	(Kiss et al., 2020)

clearance (Sturmlechner et al., 2021), which exhibit specific features of PASP. These distinctive characteristics of PASP as well as SASPs again highlight the heterogeneous impacts of senescence on malignancy. Other than cancer initiation and progression, cancer therapy effects can be affected by senescence and its associated phenotypes.

The heterogeneous role of SASPs in cancer therapy

Untransformed fibroblasts and epithelial cells were primarily investigated in early senescence research. For the past two decades, immortal and transformed cancer cells have also been leveraged to induce chemotherapy- or radiation-induced senescence, which is known as therapy-induced senescence (TIS) (Ewald et al., 2010; Hwang et al., 2020; Saleh et al., 2020). However, the molecular understanding of TIS in the cancer setting remains unclear (Perez-Mancera et al., 2014; Schosserer et al., 2017). On the one hand, TIS can prevent cancer cell growth *via* SASP-associated stimulation of the tumor growth inhibiting effect (Ewald et al., 2010). Conversely, SASP can promote resistance to cancer therapy by SASP-driven increase in cancer stemness (Faget et al., 2019). Several kinase inhibitors for cancer therapy have been implicated in inducing detrimental SASPs, which can create tumorigenic environments (Chambers et al., 2021). These paradoxical impacts of SASP in cancer therapy suggest the importance of cellular and tissue context (Prasanna et al., 2021), which hinders the ability to characterize cells under senescence. However, the *in vivo* and *in vitro* identification of senescent cells and their therapeutic use in cancer research have been limited by a lack of reliable and universally measurable biomarkers (Casella et al., 2019).

SINGLE-CELL RNA SEQUENCING

Given that cancer progression is a dynamic process that involves multiple steps from oncogenesis to the development of treatment resistance, it is critical to define the temporal and molecular nature of each step in this process (Lei et al., 2021). Although the phenotype of senescent cells is highly heterogeneous, the molecular factors responsible for such variability as well as the presence of potential biomarkers of senescent cells are poorly understood (Hernandez-Segura et al., 2017; Kim and Kim, 2021; Wiley et al., 2017). Single-cell RNA sequencing (scRNA-seq) may serve as an important tool for deciphering the role of senescent cells in cancer. Given that whole transcriptome sequencing of single cells was first reported in 2009, the number of cells profiled by single-cell analysis has increased exponentially (Tang et al., 2009). There are currently many competing scRNA-seq protocols that offer specific advantages and disadvantages (Mereu et al., 2020; Ziegenhain et al., 2017). Along with sequencing protocols, methods for analyzing scRNA-seq data have also grown rapidly with more than 1,000 tools currently developed (Zappia and Theis, 2021). Benchmarking studies comparing experimental and computational/analytical tools specifically designed for scRNA-seq data are available elsewhere (Luecken et al., 2022; Mereu et al., 2020; Zhang et al., 2020). Table 1 features selected scRNA-seq studies on cancer and aging or senescence that have been published over the last 5 years.

These studies suggest that age-related gene signatures mediate varying effects in the pathogenesis of different types of cancer (Chatsirisupachai et al., 2021; Wang et al., 2022; Zhai et al., 2022; Zhang et al., 2021). Gene expression associated with senescence exhibits significant heterogeneity that may contribute to the development of distinct tumor cell subpopulations (Gao et al., 2021; Hernandez-Segura et al., 2017). In a multiplexed scRNA-seq study profiling 198 cancer cell lines from 22 cancer types, a total of 12 expression programs associated with the cell cycle, senescence, and EMT were found to be recurrently heterogeneous within multiple cancer cell lines (Kinker et al., 2020). Another pancancer scRNA-seq study in humans reported that among 68 stromal cell populations, 46 were shared between cancer types, whereas 22 were specific for each cancer type (Qian et al., 2020). Moreover, an overall discordance between analyses of single cells versus bulk was found in terms of metabolic activity specifically higher in malignant cells, which can only be detected with gene expression profiling at the single-cell level (Xiao et al., 2019). These representative scRNA-seq studies show how single-cell level analysis can be used to describe the heterogeneous landscape and dynamics in cancers. Notably, intratumoral and cellular heterogeneity with phenotypic diversity, such as surface markers and (epi)genetic abnormalities, is a great challenge to cancer diagnosis and treatment (Prasetyanti and Medema, 2017; Qian et al., 2017). Overall, scRNA-seq is a novel technique that is improving our understanding of complex tumor heterogeneity in specific cellular subpopulations exhibiting dysregulation of gene expression associated with senescence.

TRANSCRIPTIONAL HETEROGENEITY OF SASP IN MALIGNANCY

The heterogeneity in senescent cells is context-dependent and is affected by the cell's origin, the type of injury that causes senescence, and the time since the injury occurred (Kirschner et al., 2020; Prasanna et al., 2021). Characterizing senescence heterogeneity is essential to understand its role in the development of cancer, such as tumorigenesis and TIS in cancer therapy (Prasanna et al., 2021). The expression levels of SA genes are highly heterogeneous and may have opposing effects on tumorigenesis and response to treatment. The composition and quantity of individual SASP factors secreted by senescent cells may vary between cell types and depend on stimuli, as summarized in Table 2 (Faget et al., 2019; Hernandez-Segura et al., 2017).

The complete SASP atlases of senescent human endothelial and fibroblast cells induced by radiation, atazanavir or RAS overexpression indicated that only 17 soluble SASP factors are shared among many senescent cells, even though other factors vary depending on tissue types and senescence stimuli (Basisty et al., 2020). In contrast, mesenchymal stem cells exposed to various stressors presented a mutual senescence phenotype characterized by four classes of SASP components among several phenotypes: extracellular matrix and cytoskeleton and/or cell binding, metabolic processes, redox factors and regulators of gene expression (Ozcan et al., 2016). Certain SASP factors may regulate the response to treatment,

Table 2. SASP and related gene signatures expressed in various cell types

Cell type	Highly expressed SASPs and SASP-associated genes	Cancer type	Reference
Plasma cells	CXCL2, CXCL1, IL-1β, SERPINE1, HMGA2, CDKN2A, OPTN, CDKN1B, BAG3, SUN1, AKR1B1, KDNA3	Multiple myeloma cancer	(Grainger et al., 2018; Saul and Kosinsky, 2021)
Epithelial colon cells	SLC30A10, ATF3, MXD1, CSPG2, CXCL14, MMP2, CXCL12, CSF-1	Colorectal cancer	(Saul and Kosinsky, 2021)
Liver hepatocytes	NFKBIA, LCAT, MT1F, UBB, RHOB, ESR1, ACADVL	Hepatocellular carcinoma	(Saul and Kosinsky, 2021)
Lung epithelial cells	CXCL2, OASL, JUND, RRAS, APOL3, PPARG	Lung cancer	(Saul and Kosinsky, 2021)
Epithelial cells of pancreatic duct	IGFBP3, SLC16A3, COL10A1, PKM	Pancreatic ductal adenocarcinoma	(Saul and Kosinsky, 2021; Storz and Crawford, 2020)
Fibroblasts	COL1A1/1A2/3A1, FN, MMP2, CDKN1A, SERPINE1, ACTA2, GLB1, CSPG2, CXCL14, SFRP2, VEGF, CTGF, GDF15	Esophageal cancer	(Kim and Park, 2019; Mellone et al., 2016)
Myeloid cells	CCL2, TNFα, CCL4, CXCL8, MCP-1, CDKN2A, PDGF-BB	Blood cancer	(Biavasco et al., 2021; Prieto and Baker, 2019)
T cells	Spp1, PD-L1, H2AJ, CXCR5, BCL6, CXCL1, CXCL2, VEGF, EREG, CSF-1, CXCL12	Malignant tumor	(Choi et al., 2021; Fukushima et al., 2018; Lian et al., 2020)

such as IL-1α, IL-6, TGF-β, CXCL1 and CXCL2 secreted by OIS in human fibroblasts, which induce senescence in autocrine manners and initiate senescence in adjacent cells *via* the paracrine signaling pathway (Acosta et al., 2013; Ruscetti et al., 2020). Interestingly, the same paracrine signaling is mediated by small extracellular vesicles (extracellular vesicle SASP or evSASP) released by human fibroblasts undergoing OIS and MCF7 tumor cells upon CDK4/6 inhibition (Borghesan et al., 2019). Another feature of the heterogeneity of senescent cells is reflected in their ability to engage various senescent cell anti-apoptotic pathways (SCAPs). For example, senescent endothelial cells require anti-apoptotic protein Bcl-xl proteins for their survival and thus show sensitivity to Bcl-xl inhibitors, whereas senescent APCs (adipocyte progenitor cells) are more sensitive to pantyrosine kinase inhibitors, such as dasatinib (Hickson et al., 2019; Kirkland and Tchkonia, 2017). Many TIS cells can avoid growth arrest, which can lead to cancer stemness and aggressive phenotypes (Prasanna et al., 2021).

However, a major hurdle for comprehensively understanding the function of senescence in cancer and for evaluating the efficiency of senolytics—agents that selectively induce apoptosis in senescent cells—is the absence of unique biomarkers that unequivocally detect and quantify senescent tumor cells *in vitro* and *in vivo* (Wyld et al., 2020). In addition to assessing the activity of senescence-related β-galactosidase (SA-β-gal), other biomarkers need to be developed to outline the process of senescence in normal cells (Gorgoulis et al., 2019). Mutations in pathways of senescence, such as CDKN2A and TP53, and deficiencies in apoptotic pathways (i.e., inactivation of the CD95 receptor/ligand system) are frequently involved cancer cells (Debatin, 2004; Hanahan and Weinberg, 2011; Park et al., 2021). Combined heterogeneity at the cellular and molecular levels may thus mediate the dynamics of diverse cancer cell subpopulations that exhibit deregulation of gene expression associated with age senescence (Jochems et al., 2021; Saul and Kosinsky, 2021). In this regard, transcriptional heterogeneity and diversity in senescent cells warrants further investigation into their clinical relevance in cancer (Davis-Marcisak et al., 2021; Faget et al., 2019; Nicos et al., 2020). Various immune checkpoint inhibitors (ICIs) and TIS have demonstrated their clinical benefits in cancer therapy, but 50%-75% of patients do not respond to these existing treatments (Nicos et al., 2020; Ribas and Wolchok, 2018). By understanding the complex SASP-receptor communication and molecular signaling networks supporting the expression of various novel SA genes in cancer cells, we may overcome cancer therapy resistance (Chambers et al., 2021). Recently, Saul and Kosinsky (2021) identified the expression of various senescence-associated genes in distinct cancer cell populations from mouse tissue, providing the basis for future studies on the role of senescence in the development of specific tumor types in human cells. More comprehensive comparisons are thus needed between various cancer types and their single-cell profiles to enable widespread use of single-cell technologies for biomarker discovery and therapeutic intervention.

CONCLUSION AND FUTURE REMARKS

Since its first discovery by Hayflick and Moorhead, cellular senescence has become an essential biological process that controls various pathophysiological functions and diseases, such as cancer. As noted by recent scRNA-seq studies, the paradoxical role and transcriptomic heterogeneity of various SASPs in the TME have been highlighted and discussed in this review. Given the heterogeneity of senescent cells and their diverse phenotypes, assessing their role in senolytics has been difficult due to the lack of universal biomarkers that can detect cancer cells *in vivo* and *in vitro* for cancer therapy. A comprehensive approach is thus needed to compare SA heterogeneity between cancer patients through translational research based on single-cell profiling (Fig. 2).

The transcriptional landscape of senescent cells can pro-

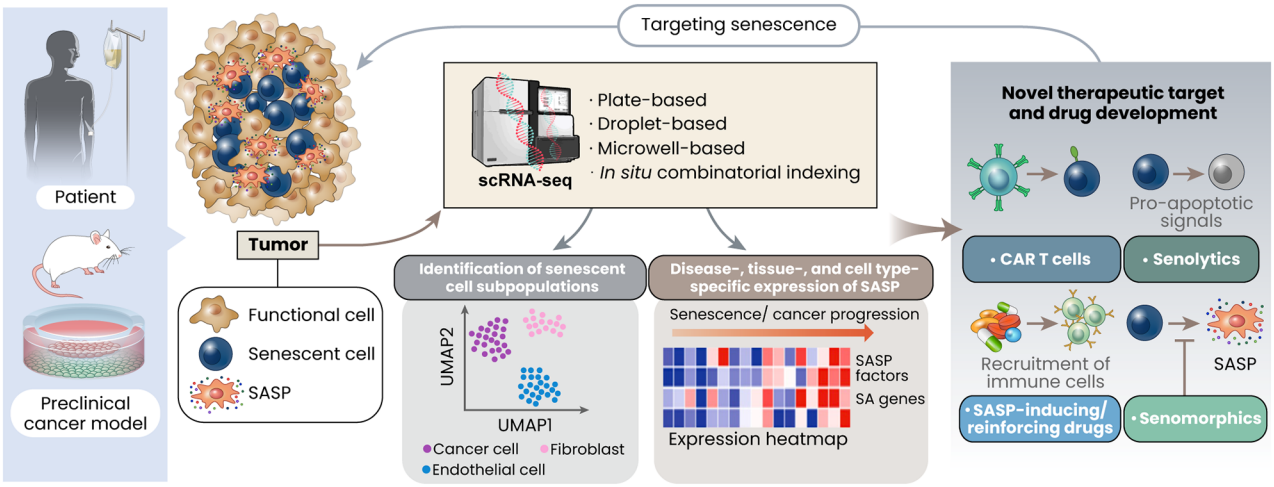

Fig. 2. Mouse to human preclinical cancer models leveraging scRNA-seq technologies play leading roles in advancing precision medicine research.

vide a thorough overview of SASP and SA gene signatures, presenting a new opportunity for the development of senotherapeutics, including senolytics and senomorphics, which are small molecules that block SASP. For example, systematic identification of antigens specific to senescent cells may facilitate the clinical development of CAR (chimeric antigen receptor) T-cell therapy, which can serve as an effective senolytic agent. Furthermore, cell–cell communication—interactions that are regulated by biochemical signaling—can be inferred from single-cell transcriptomics. This field is expanding rapidly along with the increase in publicly available scRNA-seq data. Inferring such intercellular relationships is crucial to realizing distinct populations of immune cells interacting with senescent cells. Continued development of experimental and analytical tools of scRNA-seq will thus allow us to investigate senescent transcriptomes and novel gene expression underlying cancer susceptibility.

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AUTHOR CONTRIBUTIONS

M.J., A.L., J.K., T.J.P., and S.B.L. wrote the manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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