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The role of endothelial senescence in malignant tumors

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List of abbreviations

TEC	Tumor endothelial cells
scRNAseq	Single-cell RNA sequencing
GSVA	Gene set variation analysis
OS	Overall survival
PFS	Progression-free survival
TMB	Tumor mutation burden
OIS	Oncogene-induced senescence
PICS	PTEN-loss-induced cellular senescence
TIS	Therapy-induced senescence
CDK	Cyclin-dependent kinase
ATM	Ataxia-telangiectasia mutated
ATR	Ataxia telangiectasia and Rad3-related protein
SA-β-gal	Senescence-associated β-galactosidase
SASP	Senescence-associated secretory phenotype
DEC1	Differentiated embryo-chondrocyte expressed gene-1
DCR2	Decoy death receptor 2
miRNAs	microRNAs
EMT	Epithelial-mesenchymal Transition
CXCL12	C-X-C Motif Chemokine Ligand 12
CXCR4	C-X-C motif chemokine receptor 4
CSF1	Colony stimulating factor 1
IFN-γ	Type II interferon
MHC	Major histocompatibility complex
PDCD1	Programmed cell death 1
CTLA-4	Cytotoxic T-lymphocyte associated protein 4
HAVCR2	Hepatitis A virus cellular receptor 2
IL-10	Interleukin 10
IL-6	Interleukin 6

TGF-β1	Transforming growth factor beta 1
AUC	Area Under the Curve
LASSO	Least Absolute Shrinkage and Selection Operator
TCGA	The Cancer Genome Atlas
ITGA5	Integrin Subunit Alpha 5
TGM2	Transglutaminase 2
FSCN1	Fascin Actin-Bundling Protein 1
PTEN	Phosphatase and tensin homolog
mTOR	Mammalian Target of Rapamycin
H3K9me3	Histone H3 lysine 9
Tregs	Regulatory T cells
IL-8	Interleukin 8
MMPs	Matrix metalloproteinases
TME	tumor microenvironment
VCAM1	Vascular cell adhesion molecule 1
ICAM1	Intercellular adhesion molecule 1
VE-cadherin	Vascular endothelial-cadherin
BCL-2	B-cell lymphoma 2
TIMP1	TIMP metallopeptidase inhibitor 1
D+Q	Dasatinib and quercetin
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
IκB	Inhibitor of nuclear factor kappa B
IKKα/β	IκB Kinase α/β
p38MAPK	p38 mitogen-activated protein kinases
CAFs	Cancer-Associated Fibroblasts
JAK2	Janus kinase 2
PCa	Prostate Cancer
GGO	Ground-glass opacity
pGGO	Pure ground glass opacity

LUAD	Lung adenocarcinoma
GEO	Gene Expression Omnibus
ICIs	Immune checkpoint inhibitors
PD-L1	Programmed death-ligand 1

List of publications

1. **Wu Z**, Uhl B, Gires O, Reichel CA. A transcriptomic pan-cancer signature for survival prognostication and prediction of immunotherapy response based on endothelial senescence. J Biomed Sci. 2023 Mar 28;30(1):21. doi: 10.1186/s12929-023-00915-5. PMID: 36978029 **(Relevant publication for my cumulative dissertation)**

2. Shi E, **Wu Z**, Karaoglan BS, Schwenk-Zieger S, Kranz G, Abdul Razak N, Reichel CA, Canis M, Baumeister P, Zeidler R, Gires O. 5'-Ectonucleotidase CD73/NT5E supports EGFR-mediated invasion of HPV-negative head and neck carcinoma cells. J Biomed Sci. 2023 Aug 24;30(1):72. doi: 10.1186/s12929-023-00968-6. PMID: 37620936

3. Uhl B, Haring F, Slotta-Huspenina J, Luft J, Schneewind V, Hildinger J, **Wu Z**, Steiger K, Smiljanov B, Batcha AMN, Keppler OT, Hellmuth JC, Lahmer T, Stock K, Weiss BG, Canis M, Stark K, Bromberger T, Moser M, Schulz C, Weichert W, Zuchtriegel G, Reichel CA. Vitronectin promotes immunothrombotic dysregulation in the venular microvasculature. Front Immunol. 2023 Feb 8;14:1078005. doi: 10.3389/fimmu.2023.1078005. PMID: 36845099

4. Schinke H, Shi E, Lin Z, Quadt T, Kranz G, Zhou J, Wang H, Hess J, Heuer S, Belka C, Zitzelsberger H, Schumacher U, Genduso S, Riecken K, Gao Y, **Wu Z**, Reichel CA, Walz C, Canis M, Unger K, Baumeister P, Pan M, Gires O. A transcriptomic map of EGFR-induced epithelial-to-mesenchymal transition identifies prognostic and therapeutic targets for head and neck cancer. Mol Cancer. 2022 Sep 8;21(1):178. doi: 10.1186/s12943-022-01646-1. PMID: 36076232

1. My contribution to the publication

Contribution to paper: A transcriptomic pan-cancer signature for survival prognostication and pre-diction of immunotherapy response based on endothelial senescence

As the sole first author of this publication, my work includes:

i) Data collection: Download the relevant transcriptome data and clinical information from public scRNA-seq and bulk-seq datasets available on GEO, TCGA, and corresponding websites.

ii) Data processing: Normalize and scale raw counts, dimensionality reduction and batch effect removal of scRNA-seq data. Merge and remove batch effects of bulk-seq data. Conduct pathway activity (GSVA. GSEA), cell–cell communication analysis (CellChat) and immune infiltration quantification analysis (CIBERSORT). Construction of machine learning model for predicting response of immune checkpoint blockade and compared the predictive performance of our model with six other published pan-cancer predictive models. Construction and validation of the pan-cancer endothelial senescence-related prognostic model (LASSO-cox model). Conduct meta-analysis, statistical analysis and figure preparation.

iii) Manuscript drafting: Abstract, Introduction (the majority), methods, results, Discussion (the majority), Conclusions, Figure legend.

iv) Paper revision: responding to reviewers' comments and interpreting data.

2. Introduction

2.1 Cancer epidemiology and hallmarks

Over the past few decades, cancer has emerged as a top reason of mortality worldwide, presenting substantial public health, economic, and societal challenges. According to the report of World Health Organization, there were an estimated 19.3 million individuals were diagnosed with cancer, and nearly 10.0 million succumbed to the disease globally in 2020, underscoring the pervasive nature of this disease (Sung et al., 2021). Although we've made strides in comprehending the causes of cancer, significant disparities in cancer outcomes persist across different populations (Miller et al., 2022; Sung et al., 2021). In 2020, Asia is expected to shoulder the heaviest cancer burden, with half of all global cases and 58.3% of deaths, despite comprising 59.5% of the world's population. Europe, with only 9.7% of the global population, is anticipated to account for 22.8% of total cancer incidences and 19.6% of deaths. The Americas are projected to report 20.9% of cases and 14.2% of total cancer mortalities. Notably, Asia and Africa are predicted to experience a higher proportion of cancer deaths (58.3% and 7.2%, respectively) compared to their incidence rates (49.3% and 5.7%). Interestingly, the most common cancers globally were breast (11.7%), lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach cancers (5.6%), while those with the highest mortality were lung (18.0%), colorectal (9.4%), liver (8.3%), stomach (7.7%), and breast cancers (6.9%). After delving into the complexities of cancer epidemiology and the patterns of its global impact, it becomes imperative to pivot towards the critical role of hallmarks of cancer, bridging our understanding from disease prevalence

to the nuances of individual diagnosis and treatment. Douglas Hanahan and Robert A Weinberg previously proposed that there are 10 hallmarks of cancer that describe functional properties acquired during tumor cell transformation that, together, promote which develop into aggressive tumors. Recently, Douglas Hanahan summarized four new characteristics of cancer, notably, cellular senescence is included among them (Hanahan, 2022). The hallmarks of cancer coalesce diverse complexities into a coherent scientific framework, enhancing our understanding of cancer's mechanisms and malignant evolution. This perspective's additional dimensions could further enrich this endeavor, deepening our insights into cancer biology and informing therapeutic strategies.

2.2 Cellular senescence

As a one of hallmark of cancer, cellular senescence represents a permanent halt in the cell cycle experienced by diploid cells, constraining their potential to proliferate indefinitely. Initially identified in the 1960s by Hayflick and Moorhead (Hayflick & Moorhead, 1961), this phenomenon was characterized by the observation that human diploid fibroblasts undergoing culture would attain a peak in cell divisions, subsequently inhibiting further growth. The biological clock, referred to as the "Hayflick limit," arises due to the successive shortening of telomeres with each cellular division and serves as a physiological mechanism designed to avert genomic instability and, consequently, the accumulation of DNA damage (Fumagalli et al., 2012). Besides telomere attrition telomere shortening (aka replicative senescence), an array of other stressors can also initiate senescence. These include, but are not limited to, oncogenic stress, reactive oxygen species, nutrient scarcity, and genotoxic stress brought on by

cancer treatments or even pathogens (Calcinotto et al., 2019; Lee & Schmitt, 2019). First, oncogene activation, for instance, HRASV12, prompts a growth cessation, termed OIS (oncogene-induced senescence), which was initially evidenced in vivo in 1997 (Serrano, Lin, McCurrach, Beach, & Lowe, 1997). Similarly, the depletion of tumor suppressor genes, such as PTEN (phosphatase and tensin homolog), can also promote senescence in primary prostate epithelium (Alimonti et al., 2010; Parisotto et al., 2018; Peeper, 2010), known as PICS (PTEN-loss-induced cellular senescence). The p53 pathway occupies a pivotal position in both OIS and PICS. In the context of OIS, oncogene activation results in DNA damage, which subsequently activates p53, thereby inducing senescence (Chandeck & Mooi, 2010). This mechanism contrasts with PICS, where p53 is activated through the mTOR (Mammalian Target of Rapamycin) pathway without evident DNA damage (Jung et al., 2019). In cancer, although malignant tumors possess the capability to bypass senescence, they can still be induced into a state of senescence through the use of therapeutic measures, resulting in TIS (therapy-induced senescence). Traditional anti-cancer treatments, including chemotherapy and radiotherapy, are recognized to promote senescence in normal epithelial cells and cancer cells (Hansel et al., 2021; Prasanna et al., 2021). Human cancer cells enter a senescent state when exposed to low doses of chemotherapy, but face apoptosis with higher doses. (Basu, 2022). From a mechanistic perspective, numerous chemotherapies induce DNA damage in cancer cells, initiating senescence via the activation of the interrelated p53–RB pathways, mediated through ATM–CHK2 and ATR–CHK1 kinase (Abdelgawad et al., 2021). Radiotherapy, utilized extensively for treating various cancer types, can provoke an unrepairable DNA damage response. This anticancer pathway can lead to

apoptosis and cellular senescence mediated by ATM (Ataxia-telangiectasia mutated) or ATR (Ataxia telangiectasia and Rad3-related protein) and the p53-p21 pathway (Tabasso, Jones, Jones, & Macip, 2019). Targeted therapies in patients may lead to an early onset accumulation of senescent cells related to the treatment. CDK4/6 (Cyclin-dependent kinase 4/6) inhibitors such as palbociclib, ribociclib, and abemaciclib have similar functions to p16INK4a, thereby strongly inhibiting proliferation, a hallmark of cellular senescence (Fassl, Geng, & Sicinski, 2022; Wagner & Gil, 2020). Taken together, extensive evidence suggests that cellular senescence is a complex biological process caused by intrinsic and extrinsic factors, particularly those resulting from therapeutic modalities that cause DNA damage.

2.3 Detection of cellular senescence

Common markers often utilized to indicate senescence encompass the 'gold-standard' SA-β-gal (senescence-associated β-galactosidase) reactivity, absence of the cell-cycle-associated Ki67 protein, pronounced expression of the CDK inhibitor p16INK4a (also recognized as CDKN2A), absence of DNA replication, along with both focal and global increased levels of trimethylated H3K9me3 (histone H3 lysine 9) and the induction of SASP factors (Gorgoulis et al., 2019; López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013, 2023). Moreover, senescent cells typically display a modification in cell size, adopting a smoother shape compared to proliferating cells, and demonstrate the formation of senescence-associated heterochromatin foci, lipofuscin accumulation, DNA damage foci, loss of Lamin B1, and distension of satellites associated with senescence. They express DEC1 (differentiated embryo-chondrocyte expressed gene-1)

and DCR2 (decoy death receptor 2), elevate certain miRNAs (microRNAs), and secrete a multitude of factors (Childs et al., 2017; Muñoz-Espín & Serrano, 2014; Sen, Shah, Nativio, & Berger, 2016). The aforementioned characteristics establish the gold-standard markers for identifying senescent cells and epitomize the genuine traits of senescence. However, utilizing even a combination of multiple markers doesn't reliably illustrate cells as senescent as opposed to being in long-term arrest or experiencing an irregular mode of delayed cell death. Consequently, affirming the existence of senescent cells, especially *in vivo*, proves challenging, and there persists a continuous search for a 'senescence signature'—essentially, a sturdy and sensitive array of markers or gene products that definitively categorize a cell as senescent (Casella et al., 2019; López-Otín, Blasco, et al., 2023; Sikora, Bielak-Zmijewska, & Mosieniak, 2021). Besides, concerning the function of senescence in cancer is increasingly elucidated, efforts are being undertaken to precisely identify senescent cells in individuals with cancer. There is an escalating interest in discovering new senescence markers that may also possess prognostic potential in senescence and cancer (He & Sharpless, 2017; López-Otín, Pietrocola, Roiz-Valle, Galluzzi, & Kroemer, 2023). A distinct trait of senescent cells lies in their ability to remain metabolically active, producing and emitting numerous factors that affect the tissue microenvironment in varied manners. The acquisition of this altered cellular metabolism is a pivotal aspect of the senescence phenotype, essential for executing the senescence program (Palmer, Tchkonia, & Kirkland, 2022; Wiley & Campisi, 2016). Degradation of catabolic enzymes (glycogen phosphorylase) leads to the accumulation of intracellular glycogen, resulting in reduced proliferation and simultaneous induction of senescence (Favaro et al., 2012; Oh et al., 2017). A

growing body of literature on cellular senescence metabolism shows that both glucose consumption and lactate production increase during senescence (Ross et al., 2010; Servillo et al., 2013; L. Zhang et al., 2022; Zhu et al., 2022).

2.4 Senescence in tumor microenvironment and tumor progression

Senescent cells, while generally scarce in healthy tissue, are frequently found within the tumor microenvironment throughout numerous, if not all, phases of tumorigenesis (Prieto, Sturmlechner, Goronzy, & Baker, 2023). First, for tumor cells, tumor cell senescence has long been considered a defense mechanism against cancer progression. Tumor cells in a senescent state are present in a steadfast state of cell cycle halt, underscoring the direct protective function of senescence as a safeguard against the onset and progression of tumors (Hermanns et al., 2017; Park, Choi, Kim, Kim, & Park, 2021). In a large tissue microarray analysis of osteosarcoma, declines in the senescence marker p16INK4a were associated with osteosarcoma progression and worse patient survival (Knösel et al., 2014). Several molecules or drugs have been proven to promote the senescence of cancer cells, consequently inhibiting their proliferation, Epithelial-Mesenchymal Transition (EMT), and increasing their sensitivity to chemotherapy drugs (Chou, Kaller, Jaeckel, Rokavec, & Hermeking, 2022; Hampl et al., 2013; Hermanns et al., 2017; C. Liu, Rokavec, Huang, & Hermeking, 2023). But later, with the in-depth research on senescent tumor cells, senescent tumor cells play an important role in various stages of tumor development through their secretion profile. Senescent mesothelioma cells induced by RAS(v12) secrete a large amount of SASP (senescence-associated secretory phenotype) by

activating STAT3, thereby promoting EMT (Epithelial-mesenchymal Transition) and chemotherapy resistance in mesothelioma cells (Canino et al., 2012). In thyroid cancer, *in vitro* assays highlighted that primary senescent tumor cells not only possess enhanced invasion capability compared to non-senescent tumor cells but also, through various assays, it was found that senescent tumor cells secreted CXCL12 (C-X-C Motif Chemokine Ligand 12), attracting CXCR4 (C-X-C motif chemokine receptor 4) -positive non-senescent tumor cells toward invasion sites (Kim et al., 2017). In head-and-neck squamous cell carcinoma, radiotherapy-mediated early cellular senescence can promote cancer cell radiotherapy resistance by upregulating the expression of CXCR2 and its ligands through the NF-KB pathway (Schoetz et al., 2021). In colorectal cancer, tumor cells in senescence reduced $CD8^+$ T cell penetration by producing higher amounts of CXCL12, leading to the depletion of CXCR4 in T cells and subsequently, hindering their directional migration. Moreover, CSF1 (colony stimulating factor 1) released by senescent tumor cells promotes the transformation of monocytes into M2 macrophages, thereby inhibiting $CD8^+$ T cell activation and fostering tumor expansion (Choi et al., 2021). However, senescent tumor cells have also been reported to heat up the tumor microenvironment, transforming tumors from ‘cold tumor’ to ‘hot tumor’. In a liver tumor mouse model enabling p53 suppression or reactivation, initiating p53-mediated senescence marginally reduced MDSCs (myeloid-derived suppressor cells) and neutrophils, while concurrently amplifying macrophage and $CD8^+$ T cell counts (Chen et al., 2023). The senescent tumor cells experienced substantial alterations in their cell surface proteome, leading to a minimum of two crucial results. First, they are highly sensitive to IFN-γ (type II interferon) signaling due to triggering of the IFN-γ receptor

named IFNGR1. Secondly, the senescent tumor cells enhanced the expression of MHC (major histocompatibility complex) class I molecules, further amplified by IFN-γ signaling, to escalate their antigen-presentation capability (Marin et al., 2023). In addition to tumor cell senescence, the senescence of other cells within the tumor microenvironment, like immune and stromal cells, is also pivotal in the progression of the tumor. First, senescent T cells affect both immune and tumor cells by engaging in various potential molecular processes within the tumor microenvironment, thereby fostering tumor development and progression (Z. Liu et al., 2023). Senescent $CD8^+$ T cells have been found in both orthotopic and metastatic tissues of a variety of tumors and are characterized by downregulation of costimulatory molecules CD27 and CD28 and upregulation of costimulatory factors PDCD1 (programmed cell death 1), CTLA-4 (cytotoxic T-lymphocyte associated protein 4) and HAVCR2 (hepatitis A virus cellular receptor 2) (Ferrara et al., 2021; Huff, Kwon, Henriquez, Fetcko, & Dey, 2019; W. Liu et al., 2020; Tedeschi et al., 2022). Moreover, senescent T cells release substantial quantities of IL-10 (interleukin 10), IL-6 (interleukin 6), and TGF-β1 (Transforming growth factor beta 1), prompting the induction of adaptive Tregs (Regulatory T cells) and enhancing the immunosuppressive character of the tumor microenvironments (X. Liu, Hoft, & Peng, 2020; Ye et al., 2014; Y. Zhao, Shao, & Peng, 2020). Moreover, our research group has previously shown that ageing neutrophils advance breast cancer progression by secreting an array of cytokines (Mittmann et al., 2021). Human precursor lung tumors contain macrophages displaying senescence indicators, and eliminating these senescent macrophages diminishes tumor burden and tumorigenesis by amplifying immune surveillance (Haston et

al., 2023; Prieto, Sturmlechner, Graves, et al., 2023). Secondly, stromal senescence frequently transpires in tumors and may be correlated with the advancement of the disease. Senescent fibroblasts have been demonstrated to increase proliferation of cultured epithelial tumor cells and to foster xenograft tumor growth after co-transplantation, to a greater extent than non-senescent fibroblasts did (Krtolica, Parrinello, Lockett, Desprez, & Campisi, 2001). The pro-tumorigenic mechanism of senescent fibroblasts was linked to the SASP and its constituent cytokines, notably IL6, IL-8 (interleukin 8), and MMPs (Matrix metalloproteinases), which directly propel cancer cell proliferation and metastasis (Bavik et al., 2006; Gabai, Assouline, & Ben-Porath, 2023; D. Liu & Hornsby, 2007). Moreover, senescent fibroblasts can recruit Tregs through the secretion of IL6, consequently diminishing the activation of cytotoxic CD8+ T cells and indirectly fostering tumor expansion (Ruhland et al., 2016).

2.5 Endothelial senescence

The endothelium, a highly dynamic cell monolayer lining the vascular network, spearheads and governs blood vessel formation through vasculogenesis. It plays a crucial role in sustaining blood fluidity and managing vascular tone by producing vasoactive compounds, crucial for stabilizing blood pressure and averting orthostasis (Jourde-Chiche et al., 2019; Rajendran et al., 2013). Additionally, the endothelium, while being selectively permeable, orchestrates the trafficking of macromolecules and immune cells out of the circulatory system. Endothelial cells consistently encounter numerous circulating factors and pathogenic stimuli, predisposing them to damage and resulting in compromised tissue and organ functionality through the dysregulation of blood flow and barrier function (Chesterman, 1988).

Additionally, pro-inflammatory cytokines and chemokines originating from endothelial cells convey detrimental signals to other vascular and non-vascular tissues and organs (Gimbrone & García-Cardeña, 2013; Wettschureck, Strilic, & Offermanns, 2019). A resulting aspect of endothelial harm is cellular senescence, a persistent state of cell cycle halt triggered by different stress factors, serving to inhibit the unrestrained proliferation of damaged cells and tumorigenesis (Bloom, Islam, Lesniewski, & Donato, 2023). In cancer, the microvascular endothelium fundamentally supports tumor progression and metastatic dissemination by guaranteeing a continuous nutrient and oxygen supply through the emergence of new blood vessels, indispensable for tumor growth and development (Lamplugh & Fan, 2021; Morganti et al., 2002). TECs, morphologically and structurally unique compared to regular endothelial cells and characterized by notably fenestrated walls and reduced intercellular junctions, develop multidimensionally, extending beyond a single layer within the tumor microenvironment (Cleaver & Melton, 2003; de Fraipont, Nicholson, Feige, & Van Meir, 2001). Beyond nutritional support, the endothelium also aids tumor metastasis by assisting tumor cell intravasation, a pivotal early stage in metastatic progression, during which invasive cancer cells enter the blood vessel lumen (Reymond, d'Água, & Ridley, 2013; Weis & Cheresh, 2011). Furthermore, TECs critically regulate immune cell infiltration within the tumor, thus playing a key role in modulating the TME (tumor microenvironment) and affecting tumor behavior and therapeutic response (Luo et al., 2022; Y. Zhao, Yu, & Li, 2020). Notably, alongside tumor cells in the tumor microenvironment, stromal cells, including TECs, also experience senescence, significantly influencing endothelial cell function (Gabai et al., 2023). The compromised cell connections in senescent endothelial cells may

also impact the tumor's vascular supply, altering nutrient and oxygen delivery (Abdelgawad, Agostinucci, & Zordoky, 2022). Most studies support the promotion of tumor progression by senescent endothelial cells. A characteristic trait of senescent endothelial cells involves the elevation of VCAM1 (vascular cell adhesion molecule 1) and ICAM1 (intercellular adhesion molecule 1) adhesion molecules. Additionally, senescent endothelial cells exhibit diminished expression of the adherent junction protein known as VE-cadherin (Vascular endothelial-cadherin) and a relaxation of cell-cell interactions. These alterations seem to facilitate enhanced adhesion of tumor cells to senescent TEC layers, and amplified transmigration through them, potentially facilitating in metastasis (Gabai et al., 2023; Jiang et al., 2022; Wieland et al., 2017). Subjecting breast cancer cells to conditioned media from senescent endothelial cells, or involving them in coculture or co-injection, amplified tumor cell growth and migration (Estepa‐Fernández et al., 2021; Hwang et al., 2020; Wang et al., 2020). Persistent activation of the NOTCH pathway triggered endothelial cell senescence, accompanied by the upregulated expression of chemokines and the adhesion molecule VCAM1, which facilitated neutrophil infiltration and tumor cell metastasis (Z. J. Liu et al., 2012; Wieland et al., 2017). In summary, these studies suggest that the senescence of TEC can have extensive effects through direct signaling to tumor cells and by modifying vascular structure and function. As a result, targeting senescence in TEC may emerge as a viable focal point for predicting survival and immunotherapy responses in cancer, potentially serving as a therapeutic target.

2.6 Senotherapy in cancer

Senotherapies have become an interesting area of research in the context of cancer, focusing on two principal strategies aimed at senescence: the eradication of Senescent Cells using senolytics, and mitigating the senescent cell phenotype through the application of senomorphics, which impede the SASP (Lucas, Cavadas, & Aveleira, 2023). Senolytics, embodying one of the pivotal strategies in senotherapy, constitute a class of agents crafted to directly eliminate senescent cells, demonstrating encouraging effectiveness in the realm of cancer treatment (Kirkland & Tchkonia, 2017). The most recognized senolytic agents encompass the multi-kinase inhibitor dasatinib, the BCL-2 (B-cell lymphoma 2) family inhibitor navitoclax (ABT-263), AMG-232 (an MDM2 inhibitor), and FOXO4-DRI (a FOXO4 blocker), all of which trigger p53-mediated apoptosis, along with natural compounds fisetin and quercetin, capable of inhibiting pro-survival PI3K signaling (Chaib, Tchkonia, & Kirkland, 2022; Robbins et al., 2021). ABT-263 eliminates doxorubicin-mediated senescent breast cancer and lung cancer cells by interrupting the binding of BCL-XL and BAX, thereby inhibiting tumor growth (Saleh et al., 2020). ABT263 has also been reported to eliminate TIMP1 (TIMP metallopeptidase inhibitor 1) deletion-induced senescent prostate cancer cells, leading to inhibition of prostate metastasis and increased sensitivity of prostate cancer to docetaxel (Guccini et al., 2021). FOXO4-DRI improves radiotherapy sensitivity of non-small cell lung cancer cells by clearing radiotherapy-induced senescent CAFs (Cancer-Associated Fibroblasts) (Meng et al., 2021). D+Q (Dasatinib and quercetin) or ABT-263 treatment can eliminate senescent hepatic stellate cells induced by hepatocyte-specific FBP1 deletion and inhibit tumor formation and growth of liver cancer (F. Li et al., 2020). The mechanism of action of senomorphic is

not to kill senescent cells, but to modulate SASP to reverse senescence-related phenotypes. Compounds that target epigenetic, transcriptional, and post-transcriptional regulators of SASP, such as JAK2/-STAT3 (NVP-BSK805), NF-κB (metformin), p38MAPK (SB203580), mTOR (Rapamycin), L1 (lamivudine), STING (H-151), and BRD4 (iBET762), have demonstrated a reduction in SASP production (D'Ambrosio & Gil, 2023). In prostate cancer, mTOR inhibitor Rapamycin can inhibit prostate cancer growth by inhibiting IL6 and IL1A secretion from senescent CAFs (Laberge et al., 2015). The anti-diabetic medication metformin has been validated as effective in decreasing the secretion of pro-inflammatory SASP factors by thwarting the translocation of NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells) to the nucleus and suppressing the phosphorylation of IκB (Inhibitor of nuclear factor kappa B) and IKKα/β (IκB Kinase α/β), as well as inhibiting the activation of NF-κB, thereby inhibiting the proliferation of prostate cancer (Moiseeva et al., 2013). Metformin also can suppress the SASP induced by radiotherapy in cancer cells and enhances the efficacy of radiotherapy both *in vitro* and *in vivo* (Schoetz et al., 2021). p38MAPK (p38 mitogen-activated protein kinases) inhibitor SB203580 inhibited p38MAPK-mediated SASP factor mRNA stabilization, leading to a notable reduction of a subset of SASP factors in senescent CAFs, thereby inhibiting the growth of prostate cancer, breast cancer and lung cancer (Alspach et al., 2014; Brichkina et al., 2016). Furthermore, senotherapies have been shown to enhance clinical responses to chemotherapy and immunotherapy. Senomorphic medication, such as JAK2 (Janus kinase 2) inhibitor, can also be deployed to 'reconfigure' the SASP in PTEN-null PCa (Prostate Cancer) tumors, thereby boosting chemotherapy efficacy by rejuvenating anti-cancer immune responses (Toso et al.,

2014). In a study utilizing mice with breast cancer, post-chemotherapy treatment with ABT-263 induced apoptosis, notable tumor reduction, and extended survival, additionally disclosing that cancer cells, which survive chemotherapy by entering senescence, are susceptible to eradication by senolytics (Shahbandi et al., 2020).

2.7 Application of machine learning in cancer

In today's rapidly evolving medical field, machine learning technology is increasingly becoming a key force in advancing oncology research and practice, especially in predicting tumor prognosis and immunotherapy response (Huang, Yang, Fong, & Zhao, 2020; Vougas et al., 2019; Yang, Zhao, Liu, & Huang, 2022). Traditional tumor prognosis and treatment response assessment often rely on clinical manifestations, histopathological characteristics, and population-based statistical data, and these methods may not fully capture the complex biological differences and disease heterogeneity of individual patients (Haug & Drazen, 2023; Ngiam & Khor, 2019; Rajkomar, Dean, & Kohane, 2019). Furthermore, with the rise of immunotherapy in cancer treatment, identifying which patients may benefit from such treatments and who may exhibit resistance or adverse effects has become an urgent clinical problem that needs to be solved (Gao et al., 2023; T. Li et al., 2023; Xu et al., 2021). Machine learning, as a branch of artificial intelligence, provides a highly precise and personalized approach to predicting tumor prognosis and treatment effectiveness by learning patterns and associations from large and complex data sets (Bera, Schalper, Rimm, Velcheti, & Madabhushi, 2019; Kourou et al., 2021). It utilizes various algorithms and models to reveal biomarkers and signaling pathways hidden in the genome, transcriptome, proteome, and clinical data, which may be difficult to

identify using traditional methods (Dlamini, Francies, Hull, & Marima, 2020; Subbiah, 2023). In the field of immunotherapy, machine learning can guide us in deciphering the complexity of the tumor microenvironment, including immune cell infiltration, tumor escape mechanisms, and the impact of a patient's own immune background on treatment response (Szeto & Finley, 2019; Z. Zhang, L. Chen, et al., 2022; Z. Zhang, Z. X. Wang, et al., 2022). By in-depth analysis of these multi-dimensional data, machine learning not only enhances our understanding of tumor biology, but also provides an empirical basis for clinical decision-making, allowing doctors to develop more targeted treatment plans based on patients' unique biological characteristics (Bannigan et al., 2021; Swanson, Wu, Zhang, Alizadeh, & Zou, 2023). Therefore, the application of machine learning in predicting tumor prognosis and optimizing immunotherapy is at the forefront of the digital transformation of healthcare and has the capability to profoundly improve treatment efficacy and life’s quality of cancer patients. For example, GGO (Ground-glass opacity) is recognized as an imaging hallmark indicative of incipient lung cancer. Despite the fact that the corresponding pathological attributes may not satisfy surgical benchmarks, the significance of GGO in diagnosing preliminary stages of lung cancer is undeniable. In this context, researchers have constructed a 15-gene signature pertinent to pGGO (pure ground glass opacity) via extensive transcriptome validation. This signature has been employed not only to anticipate the prognosis of initial-phase LUAD (lung adenocarcinoma) but also to delve into the immune microenvironment inherent in GGO. The prognostic precision of this distinctive gene signature for individuals with nascent-stage adenocarcinoma has been substantiated through analysis of data from TCGA and GEO (Gene Expression Omnibus) datasets (Z. Zhao et al., 2022).

Kong and associates amassed clinical outcome data coupled with transcriptomic information from over 700 patients received ICIs (immune checkpoint inhibitors). Utilizing a machine learning approach based on protein–protein interaction networks (NetBio), they predicted the response to ICI therapy in three varied cancer categories. The precision of the NetBio model outshone that of forecasts based on conventional ICI therapy biomarkers, such as PDCD1, PD-L1 (programmed death-ligand 1), or CTLA-4, and etc. (Kong et al., 2022). These studies demonstrate the important role of machine learning in cancer prognosis and treatment outcome prediction.

2.8 Objective

Cellular senescence significantly regulates the functional characteristics of cells and has recently been identified as a key feature of solid malignancies, known as the "hallmarks of cancer." Forecasting individual survival and therapy outcomes is vital for creating tailored treatment strategies in precision oncology. Given the microvascular endothelium's essential role in cancer immune biology and resistance to treatment, we theorize that the senescence of TEC plays a significant role in both tumor advancement and the effectiveness of immunotherapy for solid cancers. Therefore, it presents a valuable target for predicting survival and immunotherapy responses.

3. Summary (in Englisch)

The microvascular endothelium plays an important role in regulating the delivery of nutrients, the provision of oxygen, and the immune surveillance of cancerous cells growth, making it a key biological factor and potential therapeutic target for cancer treatment. Recent studies indicated cellular senescence was a hallmark of solid tumors. Notably, there are findings that senescent tumor endothelial cells develop a senescence-associated secretory phenotype. This phenotype undergoes a shift towards a pro-inflammatory transcriptional activity that further encourages tumor proliferation and metastasis. Based on these insights, we propose that targeting the senescence in TEC (tumor endothelial cells) could offer a valuable approach for predicting survival outcomes and the effectiveness of immunotherapy, thereby advancing personalized treatment strategies in oncology.

To explore the predictive potential of TEC senescence for tumor prognosis, we performed analyses using 18 public scRNAseq cohorts, assessing the extent of cellular senescence in distinct cell populations across multiple cancer types. For this purpose, we implemented an well-known senescence-related gene signature, *FRIDMAN.SENESCENCE.UP*, to estimate the degree of senescence in each cell type. Our transcriptomic study shows that among all cell populations in the vascular compartment of malignant tumors, TEC exhibit the most pronounced senescence. Furthermore, we developed a novel TEC-specific and senescence-associated transcriptomic signature comprising 102 genes, called *EC.SENESCENCE. SIG*. Functional enrichment analysis also revealed that pathways related to the regulation of cell adhesion were enriched in *EC.SENESCENCE. SIG*. Then, our analysis revealed that enrichment

of EC. SENESCENCE.SIG exhibited a positive correlation with numerous tumorigenic signaling pathways across all cancer types included in the TCGA cohorts. Enrichment of *EC.SENESCENCE.SIG* was related to dysregulated tumor infiltration by $CD8^+$ T cells and M2-polarized tumor-associated macrophages. elevated *EC.SENESCENCE.SIG* GSVA (Gene set variation analysis) scores corresponded with poorer OS (overall survival) and PFS (progression-free survival) in a spectrum of over ten different cancer categories.

In this study, utilizing the TCGA cohorts, we show that there is a negative correlation between EC.SENESCENCE.SIG GSVA score and TMB (tumor mutation burden). Notably, a machine learning model based on *EC.SENESCENCE.SIG* exhibited encouraging AUC (Area Under the Curve) values across different datasets. Furthermore, this model outperformed previously published transcriptomic models, offering enhanced pan-cancer predictive accuracy for immunotherapy responses. To enhance the predictive performance of *EC.SENESCENCE.SIG*, we also developed a prognostic model that employs LASSO-Cox (Least Absolute Shrinkage and Selection Operator - Cox) regression. We observed that higher risk scores were positively associated with advanced disease stages and diminished OS in pan-cancer cohorts. An association was found between the risk score and an increase enrichment in pro-tumorigenic signaling pathways in nearly all cancer types within the TCGA (The Cancer Genome Atlas) cohorts. In pursuit of heightened precision in this prognostic model, we integrated the clinical disease stage with the risk score related to *EC.SENESCENCE.SIG*. Our findings indicate that the survival forecasts based on this combined nomogram score closely match the actual survival probabilities observed over a 5-year follow-

up period. In order to enhance the practical use of *EC.SENESCENCE.SIG* in clinical settings, we employ three different machine learning algorithms to detect ITGA5 (Integrin Subunit Alpha 5), TGM2 (Transglutaminase 2), and FSCN1 (Fascin Actin-Bundling Protein 1) as crucial genes in *EC.SENESCENCE.SIG* for predicting overall survival.

To summarize, our research offers new understandings regarding the diverse molecular and cellular mechanisms linked to cellular senescence within the vascular portion of malignant tumors. From a translational standpoint, the pan-cancer gene signature *EC.SENESCENCE.SIG*, which is related to senescence in endothelial cells, could prove advantageous in predicting patient prognosis and determining their response to immunotherapy within the field of precision oncology **(Figure 1)**.

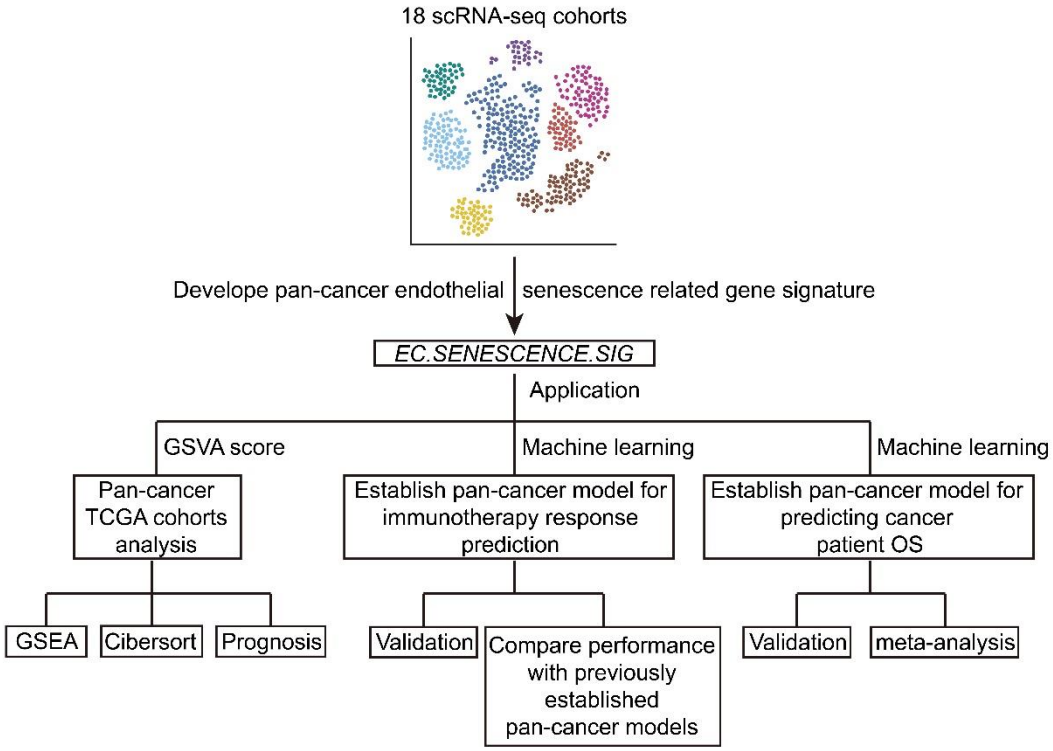

Figure 1 Research flow chart of publication for Cumulative dissertations.

4. Zusammenfassung (deutsch)

Das mikrovaskuläre Endothel spielt eine wichtige Rolle bei der Regulierung der Nährstoffzufuhr, der Sauerstoffversorgung und der Immunüberwachung des Wachstums von Krebszellen, was es zu einem Schlüsselfaktor in der Biologie und einem potenziellen therapeutischen Ziel für die Krebsbehandlung macht. Jüngste Studien haben gezeigt, dass zelluläre Seneszenz ein Kennzeichen von soliden Tumoren ist. Insbesondere gibt es Erkenntnisse, dass seneszente Tumor-Endothelzellen einen mit der Seneszenz verbundenen sekretorischen Phänotyp entwickeln. Dieses Phänotyp erfährt eine Verschiebung hin zu einer pro-entzündlichen transkriptionellen Aktivität, die das Tumorwachstum und die Metastasierung weiter fördert. Auf der Grundlage dieser Erkenntnisse schlagen wir vor, dass die Seneszenz in TEC (Tumor-Endothelzellen) einen wertvollen Ansatz für die Vorhersage von Überlebensergebnissen und der Wirksamkeit der Immuntherapie bieten könnte, wodurch personalisierte Behandlungsstrategien in der Onkologie vorangetrieben werden.

Um das prognostische Potenzial der TEC-Seneszenz für den Tumor zu erforschen, führten wir Analysen mit 18 öffentlich verfügbaren scRNAseq-Kohorten durch und bewerteten das Ausmaß der zellulären Seneszenz in verschiedenen Zellpopulationen bei mehreren Krebsarten. Zu diesem Zweck implementierten wir eine bekannte, mit Seneszenz verbundene Gensignatur, *FRIDMAN.SENESCENCE.UP*, um den Grad der Seneszenz in jedem Zelltyp zu schätzen. Unsere transkriptomische Studie zeigt, dass TEC unter allen Zellpopulationen im vaskulären Kompartiment von

bösartigen Tumoren die ausgeprägteste Seneszenz aufweisen. Darüber hinaus entwickelten wir eine neue, TEC-spezifische und mit Seneszenz verbundene transkriptomische Signatur, die aus 102 Genen besteht und als *EC.SENESCENCE.SIG* bezeichnet wird. Die funktionelle Anreicherungsanalyse ergab auch, dass in *EC.SENESCENCE.SIG* Wege im Zusammenhang mit der Regulierung der Zelladhäsion angereichert waren. Dann zeigte unsere Analyse, dass die Anreicherung von *EC.SENESCENCE.SIG* eine positive Korrelation mit zahlreichen tumorigenen Signalwegen in allen im TCGA-Kohorten enthaltenen Krebsarten aufwies. Die Anreicherung von *EC.SENESCENCE.SIG* hing mit einer dysregulierten Tumorinfiltration durch $CD8^+$ T-Zellen und M2-polarisierten tumorassoziierten Makrophagen zusammen. Erhöhte *EC.SENESCENCE.SIG* GSVA (Gene Set Variation Analysis) -Werte korrelierten mit einer schlechteren OS (Overall Survival) und PFS (Progression-Free Survival) in einer Reihe von über zehn verschiedenen Krebskategorien.

In dieser Studie zeigen wir anhand der TCGA-Kohorten, dass es eine negative Korrelation zwischen dem *EC.SENESCENCE.SIG* GSVA-Score und der TMB (Tumor Mutation Burden) gibt. Bemerkenswert ist, dass ein maschinelles Lernmodell, das auf *EC.SENESCENCE.SIG* basiert, ermutigende AUC-Werte (Area Under the Curve) in verschiedenen Datensätzen aufwies. Darüber hinaus übertraf dieses Modell zuvor veröffentlichte transkriptomische Modelle und bot eine verbesserte pan-kanzerogene Vorhersagegenauigkeit für Immuntherapie-Reaktionen. Um die Vorhersageleistung von *EC.SENESCENCE.SIG* zu verbessern, haben wir auch ein prognostisches Modell entwickelt, das die LASSO-Cox-Regression (Least Absolute Shrinkage and Selection

Operator - Cox) verwendet. Wir stellten fest, dass höhere Risikowerte positiv mit fortgeschrittenen Krankheitsstadien und einer verminderten OS in pan-Krebs-Kohorten zusammenhingen. Es wurde eine Assoziation zwischen dem Risikoscore und einer erhöhten Anreicherung von pro-tumorigenen Signalwegen in nahezu allen Krebsarten innerhalb der TCGA-Kohorten gefunden. Um die Präzision dieses prognostischen Modells zu erhöhen, integrierten wir das klinische Krankheitsstadium mit dem Risikoscore, der mit *EC.SENESCENCE.SIG* zusammenhängt. Unsere Ergebnisse zeigen, dass die Überlebensprognosen, die auf dieser kombinierten Nomogrammpunktzahl basieren, den tatsächlich beobachteten Überlebenswahrscheinlichkeiten über einen 5-Jahres-Nachbeobachtungszeitraum eng entsprechen. Um den praktischen Einsatz von *EC.SENESCENCE.SIG* in klinischen Umgebungen zu verbessern, verwenden wir drei verschiedene maschinelle Lernalgorithmen, um ITGA5 (Integrin-Untereinheit Alpha 5), TGM2 (Transglutaminase 2) und FSCN1 (Fascin Actin-Bündelungsprotein 1) als entscheidende Gene in *EC.SENESCENCE.SIG* für die Vorhersage des Gesamtüberlebens zu identifizieren.

Zusammenfassend bietet unsere Forschung neue Erkenntnisse über die verschiedenen molekularen und zellulären Mechanismen, die mit der zellulären Seneszenz im vaskulären Bereich von bösartigen Tumoren verbunden sind. Aus translationaler Sicht könnte die pan-Krebs-Gensignatur *EC.SENESCENCE.SIG*, die mit Seneszenz in Endothelzellen zusammenhängt, vorteilhaft sein, um die Prognose der Patienten vorherzusagen und ihre Reaktion auf die Immuntherapie im Bereich der Präzisionsonkologie zu bestimmen **(Figure 1)**.

5. Paper

The source of the respective publication:

Wu Z, Uhl B, Gires O, Reichel CA. **A transcriptomic pan-cancer signature for survival prognostication and prediction of immunotherapy response based on endothelial senescence**. J Biomed Sci. 2023 Mar 28;30(1):21. doi: 10.1186/s12929-023-00915-5. PMID: 36978029; PMCID: PMC10045484.

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