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Integrative Transcriptomic Analysis Decodes the Interplay Between Aging, Senescence, and Cancer

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ABSTRACT

Cancer risk increases with age, and cellular senescence may be a major contributor to cellular carcinogenesis. Enormous efforts have been made to investigate the interrelation between aging and tumors, but little is known about the comparative features of normal aging, cellular senescence, and cancer at single-cell resolution. By integrating analyses of genomics, epigenomics, and bulk and single-cell transcriptomics, we revealed a directionally opposite transcriptional profile between cellular senescence and tumorigenesis at the single-cell level, which may be affected by epigenomic regulations. A total of 648 aging-dependent senescence-associated coregulated modules (SACMs), disproportionately affecting the reproductive systems of both females and males, were initially defined across 17 tissues. Single-cell analysis revealed that aging primarily affects endothelial cells, followed by T cells, epithelial cells, macrophages, and fibroblasts. Opposite directions of change in gene expression between aging and cancer can commonly be observed in endothelial, fibroblast, and epithelial cells, which may prompt the opposing patterns of gene expression between tissue aging and epithelial carcinoma at the bulk level. A similar pattern of expression can be observed in immune cells, which are characterized by decreased self-renewal with aging, but this pattern is reversed in epithelial carcinoma. Our study highlighted the role of senescence as a natural barrier against tumor formation and supported the idea that aging-related systemic environment changes create a protumorigenic milieu.

1 | Introduction

Aging is the most important risk factor for many cancers [1]: the molecular basis behind this relationship is not fully understood, although common hallmarks, including genomic instability, heightened inflammation, and telomere attrition, have been identified [2]. The lower incidence of tumors in both larger and longer-lived mammalian species suggests that the increased incidence of cancer with aging is not simply the result of the passage of time [3, 4]. Increasing evidence has revealed that age-induced reprogramming of the microenvironment plays a major role in driving efficient tumorigenesis [5].

Abbreviations: DMGs, differentially methylated genes; DTDA, gene expression downregulation in tumors and aging; DTUA, gene expression downregulation in tumors and upregulation in aging; MEGENA, multiscale gene coexpression network analysis; ORA, overrepresentation analysis; SACMs, aging-dependent senescence-associated coregulated modules; SAS, cell-type-specific aging-dependent senescence-associated signatures; scRNA-seq, single-cell RNA sequencing; UTDA, gene expression upregulation in tumors and downregulation in aging; UTUA, gene expression upregulation in tumors and aging.

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One common microenvironment that emerges during aging is "inflammaging", a process of persistent and low-grade chronic inflammation, which has been identified as a trigger for tumor progression [6]. Age-induced immunosenescence is another factor contributing to cancer development [7]. As the most common stromal components within tissues, fibroblasts, endothelial cells, and epithelial cells played a tumorigenic role through the release of the relevant senescence-associated secretory phenotypes (SASPs) [8–10]. However, direct and comprehensive age-related studies of these cell types during tumorigenesis are limited.

Although the comparative molecular characteristics of tumors between younger and older patients, as well as the expression and coregulation of senescent genes in a variety of normal human tissues, have been characterized [11, 12], few studies have explored the relationships between molecular characteristics in normal aging and cancer [13, 14]. In addition, cell type average-dependent bulk transcriptome analysis ignores cell-type-specific functions and their distinct courses of senescence during aging; a high-dimensional cell-level analysis is needed to shed light on the connections between aging and tumorigenesis.

By integrating cellular senescence signatures with bulk and single-cell transcriptome datasets from 17 tissues, cell-type-dependent aging signatures and their associations with tumorigenesis were established in this work. Our results highlight that endothelial cells are affected the most by aging, followed by T cells, epithelial cells, and fibroblasts. A significantly decreased self-renewal capacity and a microenvironment alteration mediated by a hyperactivated collagen metabolic process were observed to occur in immune cells and stromal cells with aging and were completely reversed in epithelial carcinoma. By integrating genomics and epigenomics, we found evidence that the opposing transcriptomic changes in epithelial cells are partially affected by alterations in DNA methylation. This study therefore offered important insights at both the tissue and cellular levels into how cell-type-specific senescence affects tumorigenesis.

2 | Materials and Methods

2.1 | GTEx Bulk RNA-Seq Processing

To analyze bulk RNA-seq datasets of human normal tissues and cancers, we downloaded the raw count of RNA-seq data from the UCSC Xena [15] and listed them in Table S1. After the tissues with fewer than 20 samples were filtered and paired with the tissue of origin of the cancers from the GEPIA2 database [16], we obtained RNA-seq samples from 17 tissues and 24 cancers.

For the GTEx bulk RNA-seq data, the samples were collected from non-diseased tissues of individuals aged 20–80 years old. For each tissue, we used genes with an expression level higher than one count in more than 20% of the samples for analysis. The read count data were normalized using the variance stable transformation method to adjust for sequencing library size differences [17].

2.2 | MEGENA Coexpression Network Analysis

We applied MEGENA [18, 19] to normalized expression data from 17 tissues, constructing co-expression networks with superior sparsity and reduced redundancy compared to WGCNA, thereby minimizing false correlations and improving reliability. Briefly, we computed Pearson correlation coefficients (PCCs) for all gene pairs. The significant gene pairs were subsequently filtered by a false discovery rate (FDR) cutoff of 0.05. Significant gene pairs are initially sorted by the PCC, and their planarity is tested iteratively to generate a planar filtered network (PFN) with enhanced similarity. Multiscale cluster analysis (MCA) was performed on PFNs to identify coexpression modules of varying degrees of compactness. Multiscale hub analysis (MHA) was performed to identify hub genes highly connected in each module.

2.3 | Identification of Aging Dependent Senescence-Associated Coregulated Modules

To identify aging-dependent senescence-associated coregulated modules (SACMs), we began by extracting the first principal component of each module and computing its correlation with sample age. We subsequently enriched the age-related modules with a curated cellular senescence gene set (Table S1) to identify the SACMs. For a detailed and comprehensive description of the identification steps, please refer to the Supplementary Methods section.

Further information on the methods is available in the Data S1.

3 | Results

3.1 | Aging Disproportionately Affects the Reproductive System

Mounting evidence indicates that genes do not operate in isolation but are coregulated in a network [20, 21]. A well-established multiscale embedded gene coexpression network analysis (MEGENA) was subsequently used to define the normal aging signatures in various human tissues from 3445 healthy samples aged 20 to 79 years (Figure 1A). In total, 17,319 coexpressed gene modules were identified in 17 tissues, with an average of 1019 modules per tissue (Figure 1B), which were not affected by sample number, age distribution, or expressed genes (Figure 1A). Among these modules, 3115 coexpressed gene modules were significantly correlated with aging (Figures 1B, S1). The top tissues containing the highest number of modules correlated with aging, including the prostate, uterus, and ovary, implied that the reproductive system was affected more strongly by aging. However, the stomach was not significantly affected by aging. To define the aging-dependent senescence-associated coregulated modules (SACMs), we compared each of the aging-correlated gene coexpressed modules to 11 manually collected senescence-associated signatures [22–30] (Figure S2A, Table S2), resulting in 648 SACMs (Figure 1C, Table S3) in 16 tissues. Although an unbiased distribution of aging-positive and aging-negative modules was observed in each tissue (Figure 1B), the coexpression pattern

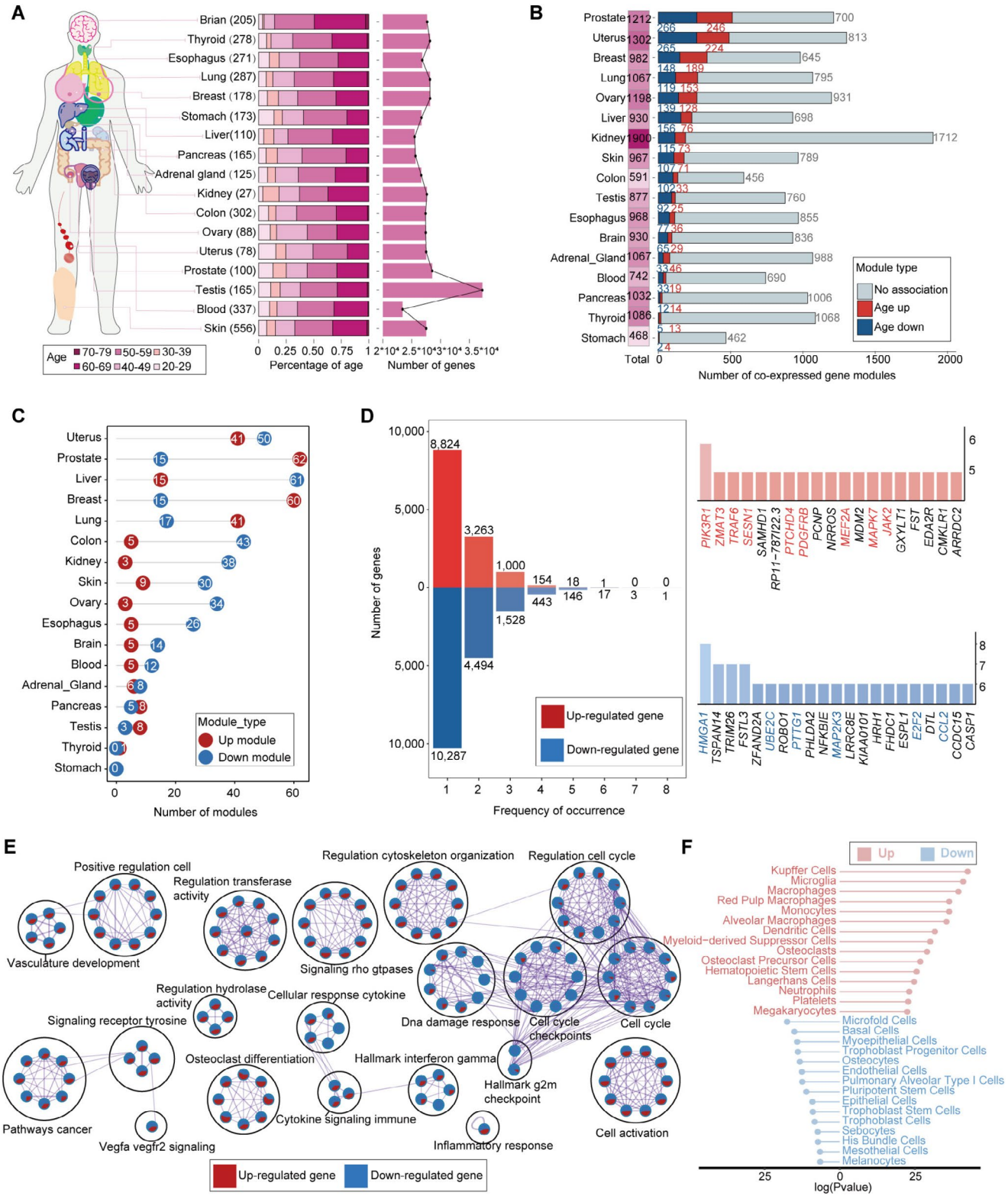

FIGURE 1 | Definition of aging-dependent senescence-associated signatures in 17 healthy tissues. (A) Statistical information, including the quantity of samples collected from 17 healthy tissues, the age range of the samples, and the number of genes used for analysis. The colors of the bar charts represent different age groups. (B) Numbers of coexpressed gene modules across 17 tissues. (C) The distribution of aging-dependent senescence-associated coregulated modules (SACMs) across tissues. (D) The distribution of shared SACM-associated genes between tissues (left) and the list of genes shared by the highest number of tissues (right). (E) Functional over-representation analysis (ORA) of positive and negative SACM-associated genes from (D) using the Metascape. Every single node represented an enriched term, and two nodes were linked if their Kappa similarities were greater than 0.3. Similar functional terms were clustered together and are displayed as circles. The node size was proportional to the number of enriched genes. Different colors represent genes whose expression is upregulated or downregulated during aging. (F) Cell types over-representation analysis of positive and negative SACM-associated genes.

between SACMs and aging differed significantly between tissues. A greater number of SACMs, found in tissues including the prostate, breast, and lung, were associated with a positive relationship. The remaining tissues with fewer SACMs, including the colon, kidney, skin, ovary, esophagus, brain, and blood, were negatively related (Figure S2B). Independent validation using WGCNA revealed significant overlap of senescence-related genes with the MEGENA results (Figure S2C), thereby confirming the robustness of our findings. This distinct number of SACMs and divergent coexpression patterns between various tissues together with the tissue-specific SACM-associated genes (Figure 1D) suggest that tissue-dependent aging progresses. However, 8.8% (1173/13260) of positive and 12.6% (2138/16919) of negative SACM-associated genes were shared by more than three tissues, suggesting that common characteristics exist between human tissues (Figure 1D). As a regulatory subunit of PI3K, PIK3R1 stabilizes but inhibits p110 activity, thereby modulating PI3K catalytic function [31]. Its age-related upregulation in six tissues (Figure 1D) impairs insulin signaling and disrupts metabolic homeostasis [32]. HMGA1, an essential structural component of senescence-associated heterochromatic foci (SAHFs) [33], shows global downregulation across eight aging tissues (Figure 1D). This decline may contribute to diminished regenerative capacity and tissue deterioration [34]. Functional overrepresentation analysis (ORA) of the common genes revealed that the cell cycle and DNA damage response-associated functions were inhibited with aging, while the inflammatory response and cytokine signaling pathways were significantly activated (Figure 1E, Table S4). However, the aging-positive genes related to SACM-associated genes were enriched in monocytes and their tissue-resident macrophages, including Kupffer cells, microglia, and osteoclasts, and the negative genes were enriched in all kinds of basal cells, including epithelial cells, fibroblasts, and endothelial cells (Figure 1F, Table S4). These findings suggest that the inhibited and activated senescence-associated functions were determined by distinct cell types.

3.2 | Unstable Microenvironment and Functional Inhibition of Epithelial Cells During Aging

The tissue-specific senescence-associated aging signatures have been defined; diverse cell types exist within each tissue, and they can display distinct molecular features during aging. To accurately delineate the cell type specificity of SACMs in each tissue, we analyzed multiple single-cell transcriptome datasets from public repositories [35–42]. This systematic approach enabled us to first identify distinct cell populations and their tissue-specific differentially expressed genes (DEGs), which were then integrated with SACMs to determine cell type-specific regulatory patterns (Figure 2A). On the basis of the analysis of single-cell transcriptome data from 15 different tissues, a total of 39 cell types were identified, including 24 tissue-specific and 15 tissue-sharing cell types (Figures 2B, S3). The kidney displayed a greater number of cell types, which corresponded to the highest module number (Figure 1B). By mapping SACMs from different tissues to different cell types, 74.38% (482/648) of SACMs, including 217 aging-positive and 265 aging-negative SACMs from 17 tissues, were enriched in 29 different cell types (Figures S4, S5A). A total of 124 SACMs were highly expressed in endothelial cells, followed by T cells, epithelial cells, macrophages, and fibroblasts (Figure 2C). All the cell types consisted of multiple tissues and were equally attributed to aging-positive and aging-negative SACMs, except for epithelial cells, which were exclusively composed of aging-negative SACMs (Figure 2D).

We prefer the common variation of a single cell type between various tissues to determine its fundamental response to aging; then the genes shared by more than two tissues by SACM-associated genes were extracted and regarded as the cell-type-specific aging-dependent senescence-associated signatures (SAS) (Figure S5B, Table S5). A functional ORA for each of the cell-type-specific SAS was performed. As expected, a senescence-associated secretory phenotype (SASP), like interleukin-4 and interleukin-13 signaling, was enriched in aging-positive endothelial cell SAS. Concordant with the increased ratio of endothelial cells in aging disease [37], endothelial-associated functions, including cell migration, vascular development, and endocytosis, are significantly enhanced with aging, indicating that senescent endothelial cells are highly reactive, secretory, and proinflammatory and have an aberrant morphological phenotype [43]. Consistent with the hyperactivated status of endothelial cells, the aging-positive fibroblast SAS was significantly enriched in extracellular matrix organization, collagen metabolic pathways, and epithelial-mesenchymal transition, which may lead to altered tissue architecture. In contrast to endothelial cells and fibroblasts, the epithelial cell SAS showed an exclusively negative relationship with aging and enriched cell proliferation, differentiation, migration (i.e., P53 pathway, cell differentiation, cell–cell adhesion, and cell morphological), cell signaling (i.e., cellular responses to stress, TNFA signaling via NFKB), and cell metabolism (i.e., positive regulation of hydrolase activity and small molecule biosynthetic processes). These findings indicate that the senescence of epithelial cells precipitates alterations in their cellular functionality and architecture, consequently diminishing the robustness of the tissue (Figures 2E, S5B, Table S6). The activated inflammatory response mediated by T cells and the inhibited phagocyte ability of macrophages suggest a diverse immune senescence phenotype during aging. However, a commonly decreased cell proliferation ability, which is characterized by downregulated cell cycle-associated functions, was observed in T cells and mast cells, revealing that immune cell self-renewal was significantly decreased during aging (Figures 2E, S5B, Table S6). Collectively, the reactivation of endothelial cells, fibroblasts, and T cells and the inhibition of immune cell proliferation reflect that senescent cells constitute an unstable microenvironment that easily collapses.

3.3 | Opposite Directions of Changes in Gene Expression Between Aging and Tumorigenesis

Aging is a significant risk factor for cancer development, with molecular alterations in senescence influencing the progression of cancer. To explore how these genes in SACMs change in cancer, we collected 7681 samples of 24 cancer patients aged 10–99 years from the TCGA database (Figures 3A, S6A,B). First, we examined the expression of the epithelial cells SAS between tumor and normal tissues and found that all five genes were commonly upregulated in all kinds of cancers (Figures 3B, S6C). To further

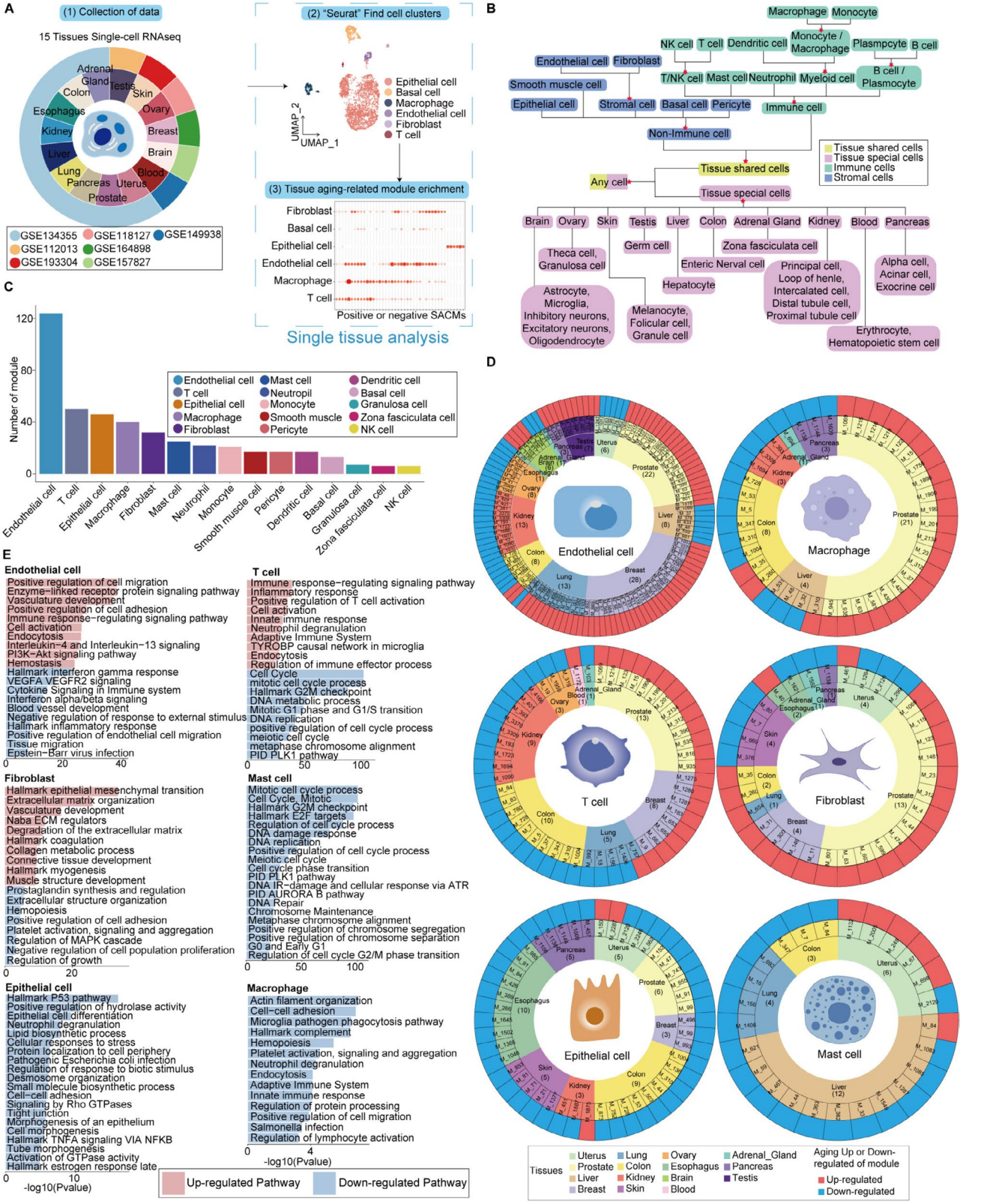

FIGURE 2 | Identification of the cell-type-specific identity of SACMs. (A) Schematic depicting the identification of cell-type-specific SACMs. The inner circle color in the data representation indicates the tissue type, the outer circle color denotes the GEO dataset, and the number represents the sample count for each dataset. (B) Hierarchical structure of the cell types across various tissues. (C) Bar plot showing the top 15 cell types mapped to the SACMs. The colors represent different cell types. (D) The top 6 cell types among all comprised aging-positive and aging-negative SACMs from multiple tissues. The inner circle represents multiple tissues, the middle circle represents SACMs, and the outer circle represents categories of SACMs. The colors of the inner and outer circles represent different organizations and module upward and downward adjustment categories. (E) Functional overrepresentation analysis of the cell-type-specific senescence-associated signatures (SAS). Pink represents upregulated pathways, light blue represents downregulated pathways, and the length of the bar graph is proportional to the *p* value of enrichment.

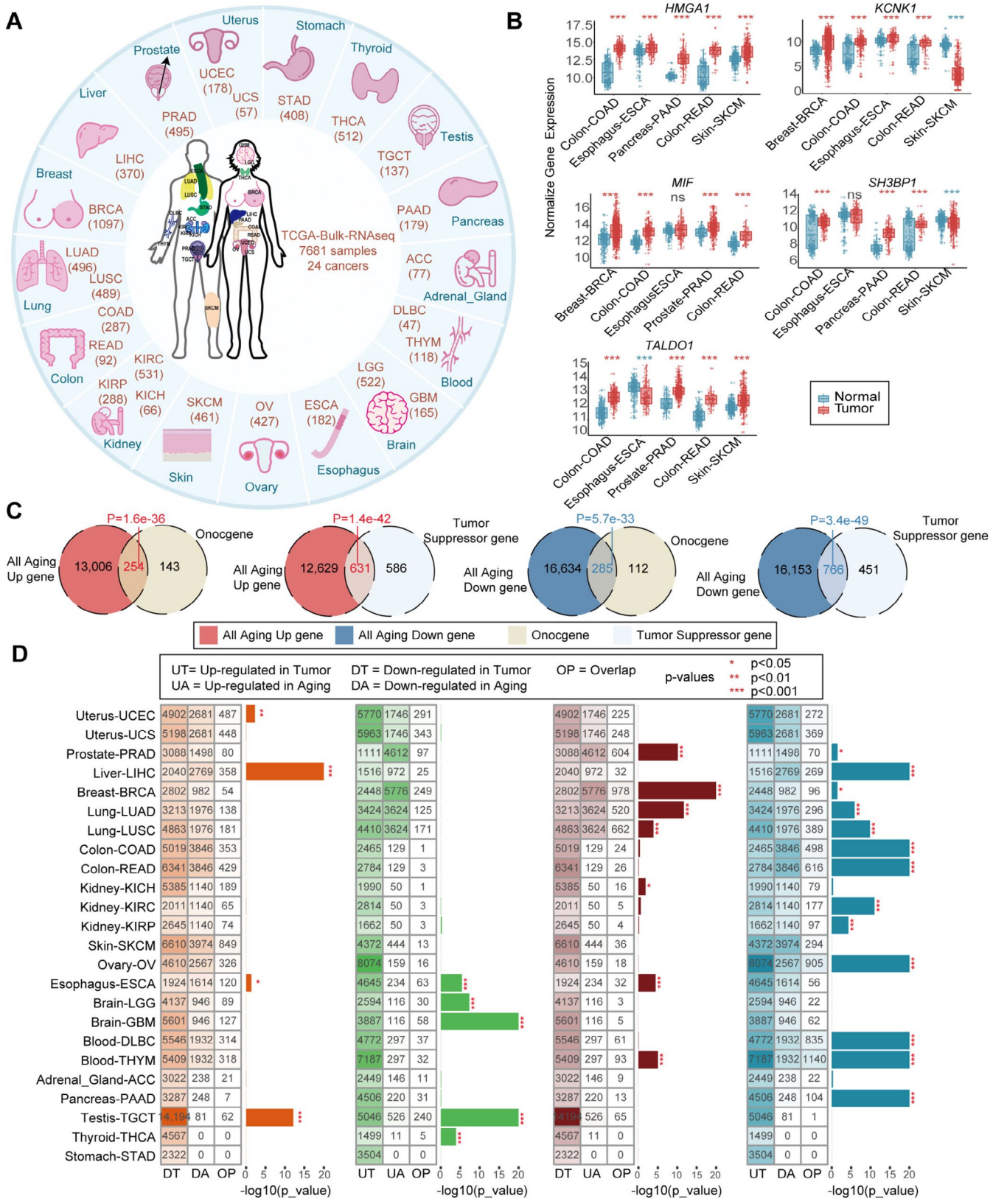

FIGURE 3 | Aging and tumorigenesis were associated with opposite trends at the tissue level. (A) Statistical information, including the quantity of samples collected from 24 cancers in the TCGA database. (B) Violin plots displaying the gene expression of five aging-negative epithelial cell SAS between tumor tissues and normal tissues. Asterisks indicate the significance level of differences. ns, not significant. (C) Overlap of cancer-suppressing genes, oncogenes, aging-positive genes, and aging-negative genes. (D) Tissue-dependent comparison of the four categories of cancer-DEGs and age-DEGs. The four panels from left to right correspond to the four categories. Within each panel, the first column represents cancer-DEGs, the second column represents age-DEGs, and the third column represents shared genes. The bar plots represent the significance of the overlap. The colors represent different expression pattern categories.

validate the variation in oncogenes during normal aging, a total of 1217 cancer-suppressing genes from the TSGene database [23] and 397 oncogenes from the OncoKB database [44, 45] were collected, with 76% (927/1217) of the cancer-suppressing genes and 86% (342/397) of the oncogenes significantly changing with aging (Figure 3C). Taken together, these findings suggest that genes whose expression is altered during senescence are also altered during tumorigenesis.

Cancer-associated differentially expressed genes (cancer-DEGs) were subsequently identified in all 24 cancer types by integrating their paraneoplastic samples from the TCGA cohort and normal tissue samples from the GTEx cohort (Figure S6D). Tissue-dependent comparisons between cancer-DEGs and aging-DEGs (genes from SACMs) were performed, and four categories, namely, DTDA (down in tumors and down in aging), UTUA (up in tumors and up in aging), DTUA (down in tumors and up in aging), and UTDA (up in tumors and down in aging), were defined. In total, 13 tumor types belong to the UTDA category. Seven tumor types are characterized by DTUA. Only five tumor types and four tumor types displayed a consistent pattern between aging and tumorigenesis categorized with UTUA and DTDA (Figure 3D). The bias of opposite regulatory patterns between tumors and aging further validates that cellular senescence is a natural barrier to tumorigenesis and, conversely, tumorigenesis after programmed senescence has broken. One special feature is that testis aging significantly overlaps with the testicular germ cell tumors (TGCTs) in the accordant patterns of both DTDA and UTUA. TGCT is a classical tumor that occurs in men aged 15 to 35 years and then sharply decreases with age.

We further divided the tumor patients into young, adult, and old groups according to their age; and then compared the aging-DEGs and aging-dependent cancer-DEGs. A consistent opposite regulatory pattern between aging and tumorigenesis was observed (Figure S7), suggesting that the opposing transcriptomic profiles in cellular senescence and tumors are independent of the age stage during tumorigenesis.

3.4 | Opposing Transcriptomic Profiles in Cellular Senescence and Carcinogenesis

As the majority of the opposing changes, especially the UTDA category, between aging and tumorigenesis as well as the global aging-negative epithelial cells SAS were observed in epithelial carcinoma (Figure 2D), we speculated that the inverse UTDA pattern was determined by epithelial cells. To validate this hypothesis, the percentage of cell-type-specific SAS between tissue aging and its corresponding cancer was estimated (Figure 4A). The higher percentage (average 70%) of the cell-type-specific SAS indicated that the signatures shared between aging and tumorigenesis were determined by a cell type-dependent pattern. For example, all the DTDA and UTUA genes from the testis were derived from distinct cell types; the former was exclusively determined by germ cells, and the latter consisted of multiple cell types (Figure S8A). Among the major cell types in each of the four categories, we first focused on the epithelial cells, which constitute the cancer cells of epithelial carcinomas. As expected, aging-negative epithelial cells SAS were globally upregulated in the epithelial carcinomas (Figure 4B). Additionally, we observed that functions suppressed with age are significantly enhanced across all epithelial carcinomas, characterized by systemic impairment of core epithelial cell functions including developmental regulation, migration control, and modulation of hydrolase/peptidase activities (Figure 4C and Table S7). The expression of the P53 signaling pathway, which is involved in both aging and tumorigenesis [46], significantly increased in tumors but decreased with aging, suggesting an activated stress response in tumors [47] and an inhibited DNA repair ability in aged epithelial cells [48]. The top three genes shared between epithelial carcinomas were MEX3A, PHLDA2, and SDC1 (Figure 4D, Table S7), whose expression was consistently increased in other nonepithelial tumors (Figure 4E), suggesting that the variation in aging-dependent epithelial cell SAS in tumorigenesis is a conserved characteristic.

Endothelial cells are affected by aging the most and are characterized by the greatest number of SACMs (Figure 2D), which promote tumorigenesis by angiogenesis [49]. Consistent with the reversed gene expression of epithelial cells between aging and tumorigenesis, we found that aging-positive endothelial cell SAS were significantly downregulated in tumors, especially in epithelial carcinomas (Figure S8B). Vasculature development, cell migration regulation, and endocytosis, which significantly increased with age, were completely reversed in tumors (Figure S8C, Table S8), which suggested a divergent vascular phenotype between aging and epithelial carcinomas. However, the endothelial cells from nonepithelial carcinomas displayed a consistent activation pattern (UTUA) of inflammatory response-associated functions, including the regulation of cytokine production and a positive response to an external stimulus, chemotaxis (Figure S8D, Table S8), which implied the emergence of a chronic inflammatory microenvironment of endothelial cells in both aging and nonepithelial carcinomas.

Fibroblasts, a category of stromal cells, constitute the microenvironment that supports tumor cells. Fibroblast SAS-mediated extracellular matrix organization was significantly upregulated with age across all tissues and downregulated in epithelial carcinomas (Figure S8E,F, Table S8), whereas it was enhanced in nonepithelial carcinomas (Figure S8G, Table S8). These findings suggest that aging has different effects on different types of carcinomas in terms of remodeling the microenvironment.

T cells, macrophages, and mast cells are immune cells that constitute integral components of the tumor microenvironment. The aging-negative T-cell SAS, macrophage SAS, and mast cell SAS significantly increased changes in cancers, and ORA revealed that cell cycle processes (i.e., the cell cycle, G2M checkpoint, and meiotic cell cycle) were activated in cancers of all types (Figures 4F, S9 and Table S9). In contrast, the aging-positive T-cell SAS were upregulated in 10 types of nonepithelial carcinomas, which are involved in the activation of the regulation of phagocytosis, the inflammatory response, and the immune response-regulation signaling pathway (Figure S9B). These findings suggest that aging organisms experience chronic inflammation and that reduced production of new immune cells results in an inability to promptly clear senescent cells, providing an opportunity for cancer cell development. Conversely, in cancer, the increased number of immune cells and inflammation may be due to the heightened immune response to the tumor.

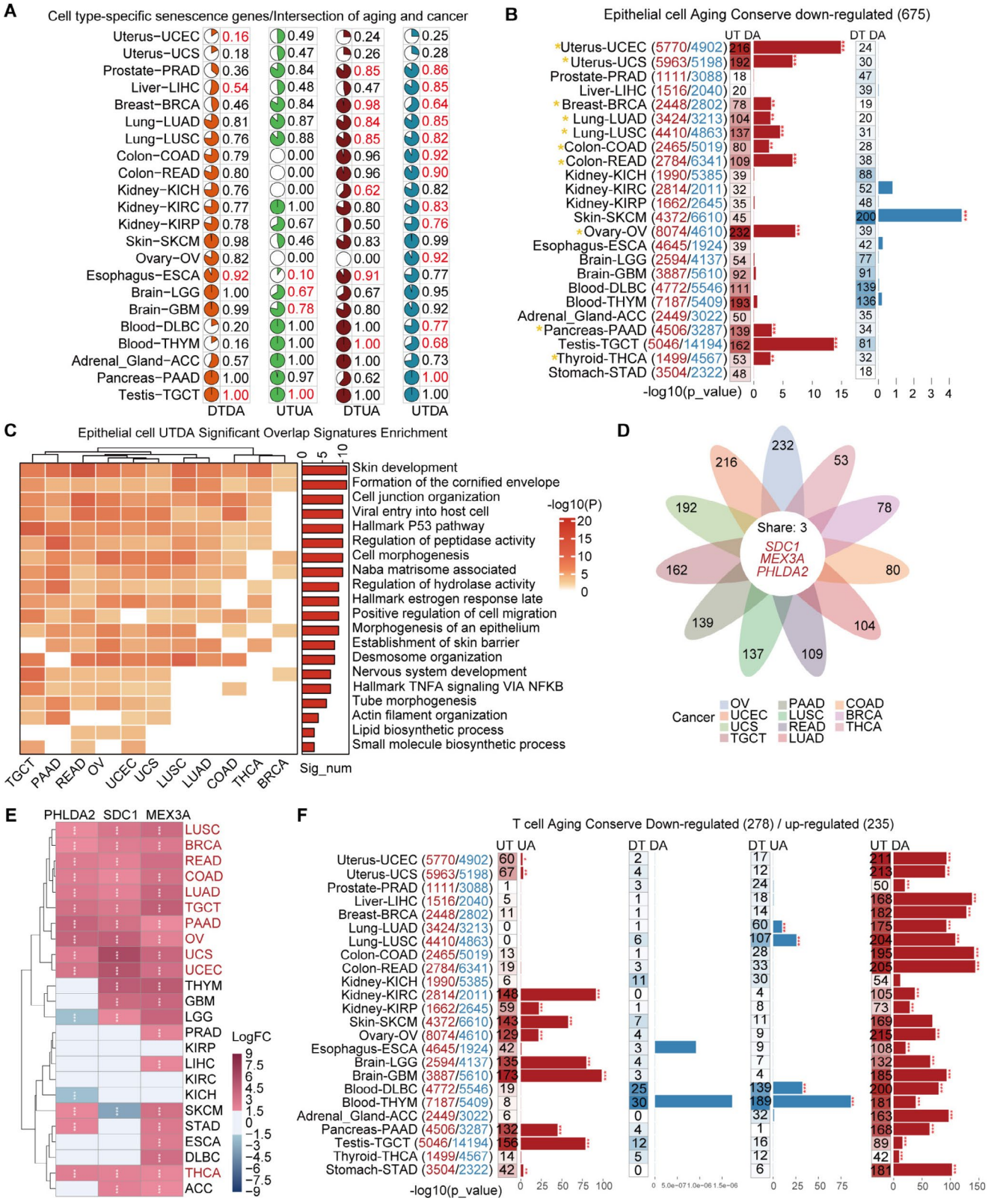

FIGURE 4 | The opposite transcriptional profile between cellular senescence and carcinogenesis at the single-cell resolution. (A) The ratio of the cell-type-specific SAS among the intersections between cancer-DEGs and age-DEGs. Pie chart colors represent different overlapping categories. Red represents significant intersections. (B) Overlap diagram of aging-negative epithelial cell SAS and cancer-DEGs across cancers. The heatmap indicates the number of overlapping genes, and the bar graph indicates the significance of the overlap. Yellow stars mark the epithelial carcinomas. (C) Functional overrepresentation analysis of UTDA pattern epithelial cell SAS. The heatmap colors represent pathway enrichment significance, white represents no restriction, white represents the opposite. The bar graph indicates the number of cancers with significant enrichment in the pathway. (D) Flower plot showing genes shared between epithelial carcinomas from the DTUA pattern. (E) Expression of the genes shared from E between tumor and normal samples. (F) Overlap diagram of aging-positive and aging-negative T-cell SAS and cancer-related DEGs across cancers.

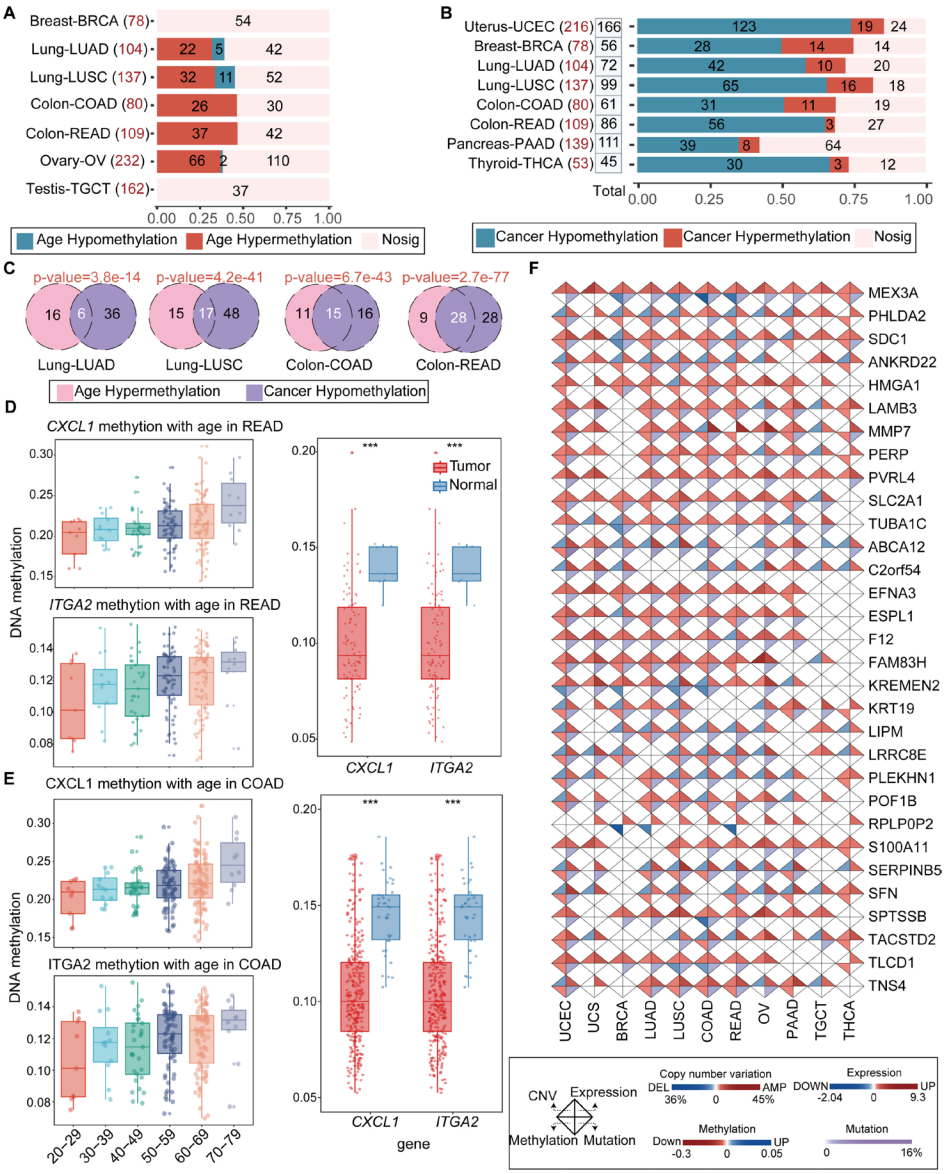

FIGURE 5 | Epigenetic and genomic alterations of epithelial related senescence associated-signatures (epithelial cell SAS). (A) Statistics of the ratio of aging-associated epithelial cell SAS with altered methylation to the total number of aging-negative epithelial cell SAS in five normal tissues. (B) Statistics of the proportion of cancer-associated methylation epithelial cell SAS among the total number of epithelial cell SAS that were upregulated in eight epithelial carcinomas. (C) Venn diagrams displaying the genes shared between genes with age-associated hypermethylation from A and genes with cancer-associated hypomethylation from B. (D, E) Tukey boxplots for the age-dependent (left) and cancer-normal (right) DNA methylation levels of CXCL1 and ITGA2 in the Colon-READ (D) and Colon-COAD (E). The colors represent different age groups. Asterisks indicate the significance level of diferences. (F) Multi-omics analysis of the top epithelial cell SAS. Each diamond is made of four pieces.

3.5 | DNA Methylation Changes, Somatic Mutations, and Copy-Number Alterations in UTDA Epithelial Cell SAS

A recent study revealed that epigenetic changes and genomic instability are hallmarks of aging that promote oncogenesis [50]. We hypothesized that the reversal of UTDA epithelial cell SAS expression in aging and cancer may be influenced by alterations in DNA methylation. To validate this hypothesis, age- and cancer-associated differentially methylated genes were identified using DNA methylation data from GTEx and TCGA (Methods) and were defined as age-DMGs and cancer-DMGs, respectively. The results revealed that aging was negatively associated with epithelial cell SAS, from the lung, colon, and ovary, which were significantly hypermethylated during aging (Figure 5A). Additionally, the activated epithelial cell SAS showed significant hypomethylation in the eight tumor samples (Figure 5B). Tissue-dependent comparisons between cancer hypomethylation and age-related hypermethylation were performed in four tissues and their corresponding cancers (lung-LUAD, lung-LUSC, colon-COAD, colon-READ) and revealed a substantial overlap (Figure 5C, Table S10). Colon-COAD displayed the greatest overlap between hypomethylated cancer-DMGs and hypermethylated age-DMGs, which are involved in the NFKB signaling pathway and the PIK3CA signaling pathway (Figure S10A). Chemokine ligand (CXCL1) and integrin subunit alpha (ITGA2), which are associated with the UTDA epithelial cell SAS, presented downregulated expression and

hypermethylation in normal aging tissues but upregulated expression and hypomethylation in cancer tissues, as observed in Colon-READ (Figure 5D) and Colon-COAD (Figure 5E). The consistent opposite patterns in gene expression and methylation further support the role of DNA methylation in influencing the transcriptome characteristics of epithelial cells during both aging and tumorigenesis.

To gain insight into the genomic characterization of epithelial cell SAS in cancer, the copy number variation (CNV) across 11 epithelial carcinomas was estimated. Limited epithelial cell SAS-associated genes presented CNVs within lung cancer (LUAD, LUSC) and four types of Pan-Gyn cancers (BRCA, OV, UCEC, and UCS) (Figures 5F, S10B and Table S11). In addition to CNV, limited somatic mutations were identified among the epithelial cell SAS (Figures 5F, S10C and Table S11). Overall, we postulated that the alterations in the methylation of epithelial cell SAS observed in these epithelial cancers may significantly impact the transcriptomic changes that occur with aging and the development of cancer.

4 | Discussion

This study presents a comprehensive landscape of aging-related alterations during cancer development from the tissue level to the cellular level. Through comprehensive gene network analysis, we characterized aging-dependent senescence-associated signatures in 17 healthy tissues and revealed that the reproductive system, comprising the prostate, uterus, and ovaries, potentially exhibits heightened susceptibility to the influence of aging. Reproductive senescence progresses more rapidly than somatic senescence does For example, menopause in females aims to halt reproductive function, thereby hastening reproductive aging [51, 52]. Consistent with the characteristics of cellular senescence [53], the biological functions of tissue-independent SACM-associated genes are related to the regulation of various aspects of cellular senescence, including the cell cycle, apoptosis repression, and SASPs. Interestingly, the genes exhibiting age-related alterations in the disease-free samples were notably enriched in several protumorigenic pathways, including vasculature development, Vegfa/Vegfr2 signaling, and cancer pathways (Figure 1E), which coincide with aging as a "driver" of cancer [54]. That is, fundamental aging processes (i.e., changes in body mechanisms with age) contribute to a procarcinogenic environment that drives the emergence of many diseases and cancers.

Alterations in gene expression typically affect the levels of their encoded proteins, thereby modulating biological functions. These tissue-conserved aging-related genes play a crucial role in the aging process. We focus on discussing the functions of these genes in aging and their biological significance. PIK3R1 encodes the phosphatidylinositol 3-kinase (PI3K) regulatory subunit p85α, which regulates PI3K catalytic activity. As a key regulatory subunit of class IA PI3Ks, p85α binds to catalytic subunits (p110α, p110β, or p110δ) to stabilize p110, suppress basal activity, and enable signal-dependent activation via phosphorylated receptors/adaptors [31]. Additionally, PIK3R1 facilitates PI3K signaling by interacting with insulin receptor substrates (IRS) and growth factor receptors, critically influencing metabolic homeostasis [32]. HMGA1 proteins accumulate on senescent cell chromatin, serving as structural components of SAHFs and stabilizing the repression of proliferation-associated genes [33]. HMGA1 is a well-established regulator of gene activation and cell proliferation. It is abundantly expressed in both embryonic and adult stem cells, where it critically maintains pluripotency, self-renewal capacity, and regenerative potential through chromatin remodeling. In contrast, HMGA1 expression is diminished or silenced in differentiated cells. Both murine and human studies demonstrate that HMGA1 expression levels decrease with age in adult stem cells, which may underlie the progressive loss of tissue regenerative capacity and age-related functional deterioration [34]. The progressive decline in the expression of senescence-associated regulators such as HMGA1 during aging highlights their essential role in maintaining cellular homeostasis, and this transcriptional downregulation may drive both aging and age-related diseases. These senescence-associated genes may be involved in core cellular processes, such as DNA repair, protein stability, energy metabolism, or anti-oxidative stress. Their age-related decline likely drives cellular dysfunction, and mechanistic studies could reveal novel targets for anti-aging interventions.

High-resolution single-cell data were subsequently employed to delineate the distinct cell types within each tissue, revealing unique molecular signatures during the aging process. Epithelial cells, which are integral to tissue integrity, delineate the boundary between the internal and external milieus [55]. These genes are strongly negatively correlated with aging, concomitant with structural and functional alterations (Figure 2C,E). This finding is in accordance with previous reports that epithelial cell aging disrupts these equilibrium states, perturbing the tissue microenvironment and fostering a milieu conducive to cancer development in cells harboring deleterious and oncogenic mutations [56]. Consistent with the concept that "a man is as old as his arteries", we observed that endothelial cells demonstrated the highest degree of cellular senescence, concomitant with increased cell adhesion, migration, and a hypersecretory phenotype (Figure 2C,E). The positioning of endothelial cells at the critical interface between circulating blood and semisolid tissues results in their continuous exposure to various circulating factors and stress stimuli [43]. Consequently, endothelial cells may experience heightened aging-related stresses, including inflammation and oxidative stress [57]. Moreover, prior research has indicated that senescent endothelial cells contribute to increased vascular permeability, facilitating cancer cell dissemination [58]. These findings underscore the importance of aging as a pivotal risk factor for cancer onset, as molecular alterations occurring during the aging trajectory can profoundly influence carcinogenesis.

The biological mechanisms underlying the link between aging and cancer are still unclear. In our research, conclusions for comparisons between age-DEGs (genes from SACMs) and cancer-DEGs (Figures 3D, S7) highlighted the opposite tissue-specific directions of transcriptomic changes between aging and cancer. One possible explanation for this phenomenon is that molecular changes associated with aging lead to an altered tissue microenvironment (hypoinflammatory, immunosuppressive microenvironment), which reduces tissue robustness and may provide a suitable microenvironment for cells that accumulate oncogenic mutations to contribute to full cancer transformation.

For example, cell morphogenesis variation in the epithelium, inhibition of P53 signaling pathways in epithelial cell senescence, enhanced vascular development of endothelial cells, and hypersecretion of ECM by fibroblasts jointly elicit tumor-promoting effects. Furthermore, immune cell senescence leads to a decline in immune function characterized by inhibited cell proliferation. This suggests a failure to clear senescent cells promptly owing to deficient immunosurveillance, which slowly accumulates and may lead to a chronic inflammatory state of the tissues as well as alterations in the microenvironment [59].

Epigenetic alterations are a meta-hallmark of cancer and aging [50]. Multiple studies have identified a variety of epigenetic changes, particularly altered DNA methylation patterns, that promote aging and cancer progression by affecting gene expression and other cellular processes [11, 60–62]. Here, we revealed DNA methylation changes corresponding to different expression levels of epithelial cell SAS in normal tissues and multiple cancers. Additionally, we examined these UTDA epithelial cell SAS for copy number variations and somatic mutations and detected fewer genomic alterations. These findings suggest that these epithelial cell SAS may not directly instigate cancer onset, but rather may undergo modified expression due to methylation regulation, thereby conferring distinct selective advantages in reshaping the tissue microenvironment for cancers characterized by diverse molecular alterations. Therefore, further studies employing causal inference methods or methylation editing techniques, along with investigations into the roles of transcription factor binding dynamics and chromatin remodeling in differential expression, are needed.

Despite our study employing comprehensive multi-omics analyses of multiple datasets and identifying key aging-related genes (MEX3A, PHLDA2, SDC1), experimental validation is still needed. Future efforts should combine molecular techniques (e.g., qPCR, Western blot) with functional assays to validate these SAS genes in aged versus tumor epithelial cells, which may provide deeper mechanistic insights into the biological links between aging and cancer progression.

Author Contributions

Jiale Cai: formal analysis, visualization, writing – original draft. **Deng Wu:** methodology, writing – review and editing. **Dahua Xu:** methodology. **Fujin Liu:** methodology. **Peihu Li:** visualization. **Dehua Zheng:** methodology. **Zhizhou Xu:** formal analysis. **Meng Cao:** formal analysis. **Yutong Shen:** investigation. **Nihui Zhang:** investigation. **Guanghui Tian:** investigation. **Chengyi Fan:** investigation. **Bo Wang:** project administration. **Kongning Li:** project administration. **Xiaoman Bi:** project administration, writing – review and editing.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All accession codes, unique identifiers, or web links for publicly available datasets are described in the paper. All codes are available upon reasonable request. The analysis code for this article has been uploaded to the associated website at https://github.com/DBprojects-lab/EpiSenCarcinogenesis.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Sunburst charts depicting co-expression modules for the 17 normal tissues. **Figure S2:** Identification of SACM in 17 healthy tissues. **Figure S3:** UMAP plot of single cells from the 15 normal tissues. **Figure S4:** Enrichment of SACMs and cell types across 15 normal tissues was conducted. **Figure S5:** Definition of cell type-specific senescence signatures. **Figure S6:** Genes with altered expression in senescence altered in tumorigenesis. **Figure S7:** Aging and tumorigenesis showed opposite directions in different age groups. **Figure S8:** The opposite transcriptional profile between cellular senescence and carcinogenesis in stromal cells. **Figure S9:** The opposite transcriptional profile between cellular senescence and carcinogenesis in immune cells. **Figure S10:** Genomic alterations of epithelial-related senescence-associated signatures (epithelial cell SAS). **Data S1:** Supplementary materials and methods. **Table S1:** Overview of datasets utilized in the study. **Table S2:** Meta information of cellular senescence features. **Table S3:** SACMs information for different tissues. **Table S4:** Functional and cell types over-representation analysis of positive and negative SACMs-associated signatures. **Table S5:** More than two tissues share cell-type-specific aging-dependent senescence-associated signatures (SAS). **Table S6:** Functional over-representation analysis of positive and negative SAS in different cells. **Table S7:** Functional over-representation analysis of genes showing differential expression due to aging and cancer in epithelial cells. **Table S8:** Functional over-representation analysis of genes showing differential expression due to aging and cancer in endothelial cells and fibroblasts. **Table S9:** Functional over-representation analysis of genes showing differential expression due to aging and cancer in Immune cells. **Table S10:** Epithelial UTDA genes with cancer hypomethylation and age hypermethylation in four tissues and their corresponding cancers. **Table S11:** Multi-omics (genomics, epigenomics) analysis of the top UTDA epithelial cell SAS.