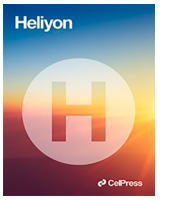
Heliyon 10 (2024) e31298

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Comprehensive analysis of gene signatures associated with aging in human aortic dissection

Jingyi Yan [a], Xuerui Tan [a,b,*]

[a] Department of Cardiology, First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, 515041, China
[b] Clinical Research Center, First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, 515041, China

ARTICLE INFO

Keywords:
Aortic dissection
Aging
Bioinformatic analysis
miRNA-mRNA regulatory network

ABSTRACT

Background: Aortic dissection (AD) is a lethal aortic disease with limited effective therapeutic strategies. Aging increases the risk of AD, yet the underlying mechanisms remain unclear. This study aims to analyze the association of aging-related genes (Args) and AD using bioinformatic analysis. This helps provide novel insights into AD pathogenesis and contributes to developing novel therapeutic strategies.

Methods: mRNA (GSE52093, GSE153434), miRNA (GSE98770) and single-cell RNA-sequencing (scRNA-seq, GSE213740) datasets of AD were downloaded from GEO database. Args were downloaded from Aging Atlas database. Differentially-expressed Args were determined by intersecting Args and differentially-expressed mRNAs of two mRNA datasets. Cytoscape was used to identify hub genes and construct hub gene regulatory networks related to miRNAs. Seurat and clusterProfiler R package were used for investigating expression patterns of hub genes at single-cell level, and functional analysis, respectively. To validate the cellular expression pattern of hub genes, the same analysis was applied to our own scRNA-seq data. Drugs targeting hub Args were determined using the DGIdb database.

Results: HGF, *CXCL8*, *SERPINE1*, *HIF1A*, *TIMP1*, *ESR1* and *PLAUR* were identified as aging-related hub genes in AD. miR-221-3p was predicted to interact with *ESR1*. A decreased *ESR1* expression in smooth muscle cell subpopulation 4 (SMC4) was observed in AD versus normal aortic tissues, which was validated by sequencing 197,605 aortic cells from 13 AD patients. Additionally, upregulated genes of SMC4 in AD tissues were enriched in the "cellular senescence" pathway. These data indicated that decreased *ESR1* might promote SMC4 aging during AD formation. Eleven existing drugs targeting hub genes were identified, including ruxolitinib and filgrastim, which are associated with AD.

Conclusions: By sequencing transcriptomic data, this study revealed aging-related hub genes and regulatory network involved in AD formation. Additionally, this study proposed a noteworthy hypothesis that downregulated *ESR1* may exacerbate AD by promoting SMC aging, which requires further investigation.

Abbreviations: AD, aortic dissection; scRNA-seq, single-cell RNA sequencing; SMCs, smooth muscle cells; SASP, senescence-associated secretory phenotype; DEMs, differentially expressed mRNAs; Args, apoptosis-related genes; Gene Ontology, GO; Kyoto Encyclopedia of Genes and Genomes, KEGG.

* Corresponding author. Department of Cardiology, First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, 515041, China.

E-mail address: doctortxr@126.com (X. Tan).

https://doi.org/10.1016/j.heliyon.2024.e31298
Received 4 July 2023; Received in revised form 19 April 2024; Accepted 14 May 2024
Available online 17 May 2024

1. Introduction

Aortic dissection (AD) is a common lethal aortic disease that needs timely treatment. However, the postoperative mortality of AD remains high, though advances have been made in treatments, especially emergent surgery [1]. In addition, approximately 20 % of patients with AD die before surgery [2]. Moreover, no effective target drugs are currently available for delaying, halting, and reversing AD formation. A better understanding of AD pathogenesis is essential for developing novel effective therapeutic strategies for AD.

AD tends to occur at the mean age of 63 years [3]. The prevalence of AD is nearly 2.5-fold higher for individuals 65–75 years of age relative to those 35–44 years of age [4,5]. Furthermore, several important features of AD are observed in the aging aorta, such as cystic medial necrosis, elastin fragmentation and fibrosis deposition [6]. However, the precise mechanisms of aging in AD pathogenesis remain unclear. The purpose of this study is to investigate the association of aging-related genes with AD at the cellular and molecular levels based on public transcriptomic data using bioinformatic analysis. The hub genes and relative regulatory network identified in this study could serve as a scientific basis for further research in AD pathogenesis and the development of novel therapies.

2. Materials and methods

2.1. Data collection

A total of 503 aging-related genes (Args) were extracted from the Aging Atlas database (https://ngdc.cncb.ac.cn/aging/index). Four transcriptomic sequencing datasets used in this study were downloaded from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) database, and see Table 1 for a detailed message. GSE52093, a microarray gene expression dataset, includes 5 normal individuals and 7 AD patients. After the removal of female subjects, 11 male individuals were included in subsequent bioinformatic analysis. The GSE153434 dataset consists of bulk mRNA-sequencing data of 10 male individuals and 10 male AD patients. A miRNA expression dataset (GSE98770) is composed of 5 samples from transplant donors and 6 samples from AD patients, and 8 male individuals were finally involved in this study after excluding female individuals. Single-cell RNA-sequencing (scRNA-seq) data of aorta derived from 3 male AD patients and 3 male donors were extracted from the GSE213740 dataset. The workflow of this study is presented in Fig. 1.

2.2. Identification of differentially-expressed aging-related genes in AD

Differentially-expressed mRNAs (DEMs) were identified via the online tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/). The cutoff value for DEMs in the GSE52093 dataset was set to an adjusted $P < 0.05$ and $|\log FC| \geq 1$, while an adjusted $P < 0.05$ and $|\log FC| \geq 1$ were the criteria for DEMs in the GSE153434 dataset. Then the DEMs of these two mRNA datasets and the Args were uploaded into the online tool Venny (https://bioinfogp.cnb.csic.es/tools/venny/) to determine the differentially-expressed Args (DEArgs).

2.3. Functional enrichment analysis of differentially-expressed aging-related genes

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis function in GeneCodis4 (https://genecodis.genyo.es/) database was applied to identify the biological function of differentially-expressed Args, and the items with $P < 0.01$ were considered significant. In brief, genes involved in significantly enriched biological processes were retrieved for KEGG pathway enrichment analysis. The genes involved in enriched pathways were then extracted for the following protein-protein interaction network (PPI) construction.

2.4. Construction of protein-protein interaction network and identification of hub genes

STRING (https://cn.string-db.org/) database was used to construct a PPI network by analyzing protein interactions. The significant gene modules of PPI were identified using the MCODE plug-in in Cytoscape (3.7.1) software. Hub genes were identified as the genes that were involved in the significant gene module.

Table 1
The characteristics of the datasets used in this study.

GSE number	Sequencing method	Platform ID	Sample type and number	Data involved in the final analysis
GSE52093	microarray	GPL10558	Tissues; 7 diseased, 5 normal	6 diseased in male, 5 normal in male
GSE153434	Bulk mRNA sequencing	GPL20795	Tissues, 10 diseased, 10 normal	10 diseased in male, 10 normal in male
GSE98770	microarray	GPL17660	Tissues, 6 diseased, 5 normal	5 diseased in male, 3 normal in male
GSE213740	Single-cell RNA sequencing	GPL18573	Tissues, 6 diseased, 10 normal	3 diseased in male, 3 normal in male

The RNA-sequencing data of males in these datasets were finally involved in the analysis. Diseased tissues: aortic tissues derived from AD.

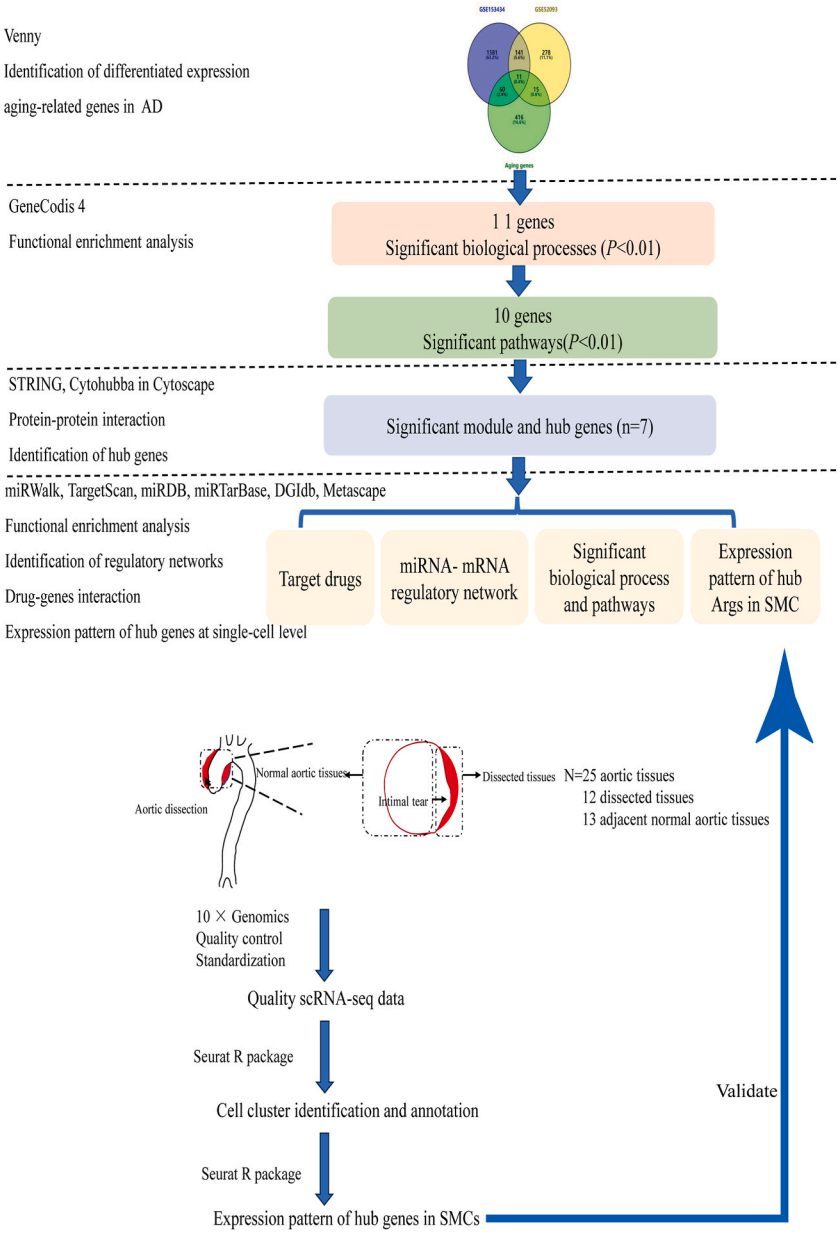


Fig. 1. Workflow chart of this study.
SMC: smooth muscle cell.

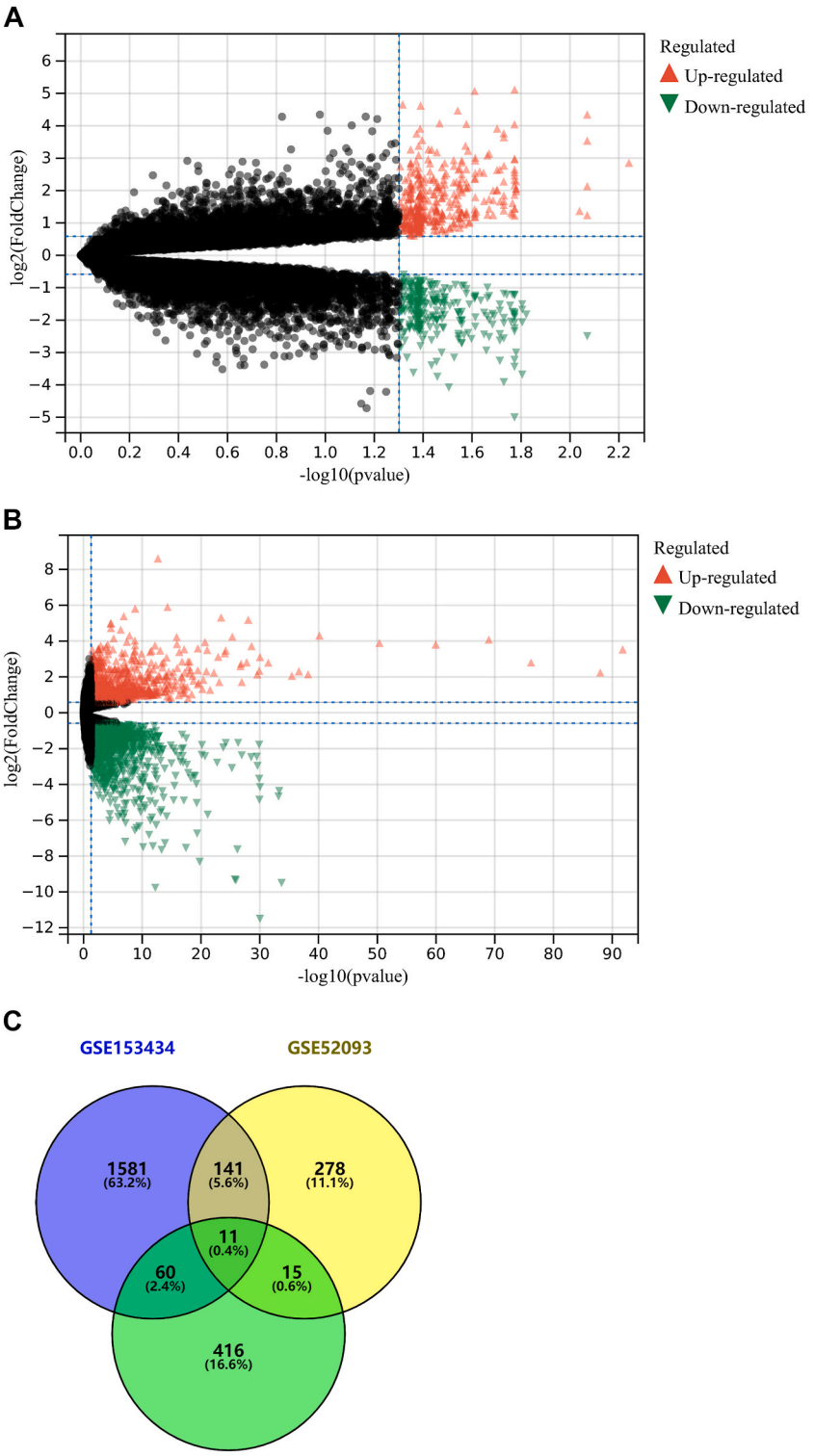


Fig. 2. Identification of the differentially-expressed aging-related genes.
(A), (B) The volcano plot of DEMs in the GSE52093 and GSE153434 datasets, respectively. (C) Venn diagram of aging-related genes and DEMs.

2.5. Functional enrichment analyses of hub genes

The biological function of hub genes was analyzed via the GO and KEGG pathway enriched analysis function in the Metascape database (https://metascape.org/gp/index.html#/main/step1). The items with $P < 0.05$ were considered significant.

2.6. Drug-gene interaction analysis

DGIdb (https://dgidb.org/) database was used to identify the potential existing drugs targeting hub genes by analyzing the interactions between drugs and hub genes.

2.7. miRNA-mRNA regulatory network

miRNAs targeting hub genes were predicted by the miRWalk, TargetScan, miRDB and miRTarBase databases. Online tool GEO2R was applied to identify differentially-expressed miRNAs (DEMis) with the criteria of an adjust $P < 0.05$ and $|\log FC| \geq 1$. The miRNA-mRNA regulatory network was constructed by integrating DEMis and the predicted miRNAs of hub genes. Cytoscape software was used to visualize the regulatory network.

2.8. Analysis of single-cell RNA-sequencing data of AD

The quality criteria for online scRNA-seq data are: Cells with UMIs$\leq$500, nGene$\leq$250 or a percentage of mitochondrial genes ($\geq$10 %) were filtered out. Subsequently, data were normalized using the LogNormalize function in the Seurat R package. FindAllMarkers function in Seurat R packages was used to identify differentially expressed genes of intra-cluster, with min. pct and logfc.threshold set to 0.25. $|\log 2FoldChange| > 0.6$ and adjusted P value < 0.05 were set as the cutoff values for differentially expressed genes of inter-clusters, and of intra-clusters between dissected and normal aortic tissues. Cell clusters were annotated based on the canonical cell markers. Seurat R package was applied to analyze the expression of hub genes at the single-cell level. ClusterProfiler and ggplot2 R package were used for functional analysis and visualization respectively. To validate the results of online single-cell data, we also analyzed our scRNA-seq data from 13 patients with AD undergoing aortic replacement surgery. Dissected tissues and nearby normal aortic tissues were collected. Single-cell suspension of the aorta was prepared by the enzymolysis protocol and then processed to construct the sequencing libraries using the 10 $\times$ Genomics Single Cell 3' v3 RNA-seq kit. The libraries were finally sequenced on the Illumina NexSeq 6000 platform. Cells with a unique molecular identifier (UMI) <400, UMI>the mean value + 2-fold of standard deviation, and the mitochondrial RNA percentage over 10 % were filtered out. Genes expressed in less than 1 % of cells would be removed. The "LogNormalize" function was used to normalize data. Principal component analysis was performed to reduce the dimensionality of data. Using a graph-based clustering algorithm, data were clustered into distinct cell clusters with distinct expression characteristics. The same analysis was applied to our scRNA-seq data to access the expression level of *ESR1*.

3. Results

3.1. Identification of differentially expressed aging-related genes

A total of 502 and 1794 DEMs were identified in GSE52093 and GSE153434 datasets respectively (Fig. 2A and 2B). As shown in Fig. 2C, 11 differentially expressed Args were identified, including 9 upregulated and 2 downregulated Args (Table 2).

Table 2
The characterization of 11 differentially expressed aging-related genes.

Genes	State
AGTR1	Downregulated
ESR1	Downregulated
CXCL8	Upregulated
HGF	Upregulated
HIF1A	Upregulated
IL1R1	Upregulated
ITGA2	Upregulated
MT1E	Upregulated
PLAUR	Upregulated
SERPINE1	Upregulated
TIMP1	Upregulated

Note, that all the genes listed in this table with an adjusted $P < 0.05$.

Table 3
Significant biological processes of the differentially expressed Args.

Biological process	Genes in query Set	*P*-value	Genes
Positive regulation of angiogenesis	4	0.0005	*HGF, SERPINE1, HIF1A, CXCL8*
Connective tissue replacement involved in inflammatory response wound healing	2	0.0005	*HIF1A, TIMP1*
Regulation of inflammatory response	3	0.0021	*ESR1, AGTR1, IL1R1*
Positive regulation of inflammatory response	3	0.0021	*AGTR1, SERPINE1, ITGA2*
Signal transduction	7	0.0026	*ESR1, AGTR1, HIF1A, PLAUR, CXCL8, IL1R1, TIMP1*
Response to muscle activity	2	0.0026	*HIF1A, ITGA2*
Negative regulation of growth	2	0.0026	*MT1E, HIF1A*
Positive regulation of nitric-oxide synthase activity	2	0.0040	*ESR1, HIF1A*
Negative regulation of extrinsic apoptotic signaling pathway via death domain receptors	2	0.0044	*HGF, SERPINE1*
Negative regulation of apoptotic process	4	0.0055	*HGF, HIF1A, PLAUR, TIMP1*
Positive regulation of DNA binding	2	0.0055	*ITGA2, PLAUR*
Positive regulation of epithelial cell migration	2	0.0055	*HIF1A, ITGA2*
Cellular response to estradiol stimulus	2	0.0063	*ESR1, ITGA2*
Epithelial to mesenchymal transition	2	0.0078	*HGF, HIF1A*
angiogenesis	3	0.0087	*SERPINE1, HIF1A, CXCL8*
Regulation of cell adhesion	2	0.0087	*PLAUR, CXCL8*
Positive regulation of interleukin-1-mediated signaling pathway	1	0.0087	*IL1R1*
Positive regulation of neuron projection regeneration	1	0.0087	*HGF*
Regulation of single stranded viral RNA replication via double stranded DNA intermediate	1	0.0087	*CXCL8*
Positive regulation of odontoblast differentiation	1	0.0087	*SERPINE1*
Positive regulation of leukotriene production involved in inflammatory response	1	0.0087	*SERPINE1*
Positive regulation of collagen binding	1	0.0087	*ITGA2*
Phospholipase C-activating G protein-coupled receptor signaling pathway	2	0.0094	*ESR1, AGTR1*

Note: Biological processes are listed in descending order of significance.

Table 4
Significant pathways of the DEArgs involved in enriched biological processes.

Pathways	Genes in the query set	*P*-value	Genes
Proteoglycans in cancer	5	0.0004	*HGF, ESR1, HIF1A, ITGA2, PLAUR*
Pathways in cancer	6	0.0015	*HGF, ESR1, AGTR1, HIF1A, ITGA2, CXCL8*
AGE-RAGE signaling pathway in diabetic complications	3	0.0091	*AGTR1, SERPINE1, CXCL8*
HIF-1 signaling pathway	3	0.0091	*SERPINE1, HIF1A, TIMP1*

Note: Pathways were listed in descending order of significance.

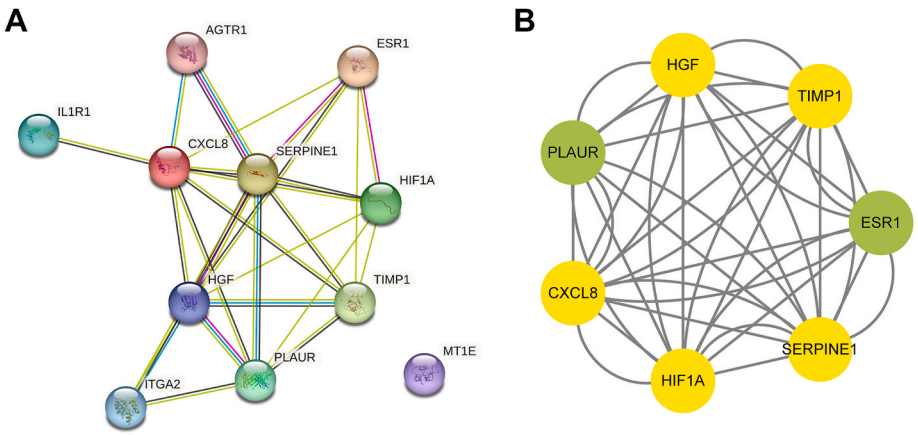


Fig. 3. Identification of hub genes.
(A) PPI network of differentially expressed aging-related genes. (B) Significant gene module obtained from the PPI network. Genes involved in this gene module were considered hub genes. Color represents the MCODE score, nodes in yellow indicate a lower score and nodes in green indicate a higher score.

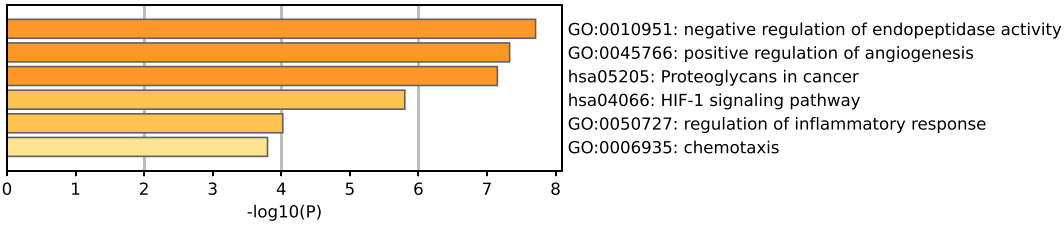


Fig. 4. Functional enrichment analysis of hub genes.
The color of the bars was proportional to the enrichment degrees.

3.2. *Functional enrichment analysis of differentially-expressed aging-related genes*

As shown in Table 3, 23 significantly enriched biological processes of 11 differentially expressed Args were identified. The top 5 biological processes were "positive regulation of angiogenesis", "connective tissue replacement involved in inflammatory response wound healing", "regulation of inflammatory response", "positive regulation of inflammatory response" and "signal transduction". KEGG pathway enrichment analysis identified 4 significant pathways with 11 differentially-expressed Args (Table 4), such as "AGE-RAGE signaling pathway in diabetic complications" and "HIF-1 signaling pathway".

3.3. *PPI network construction and hub gene identification*

With the minimum required interaction score set to 0.4, a PPI network with 10 nodes and 52 edges was constructed based on 11 differentially expressed Args (Fig. 3A). Only one significant gene model with 7 nodes and 40 edges was identified (Fig. 3B).

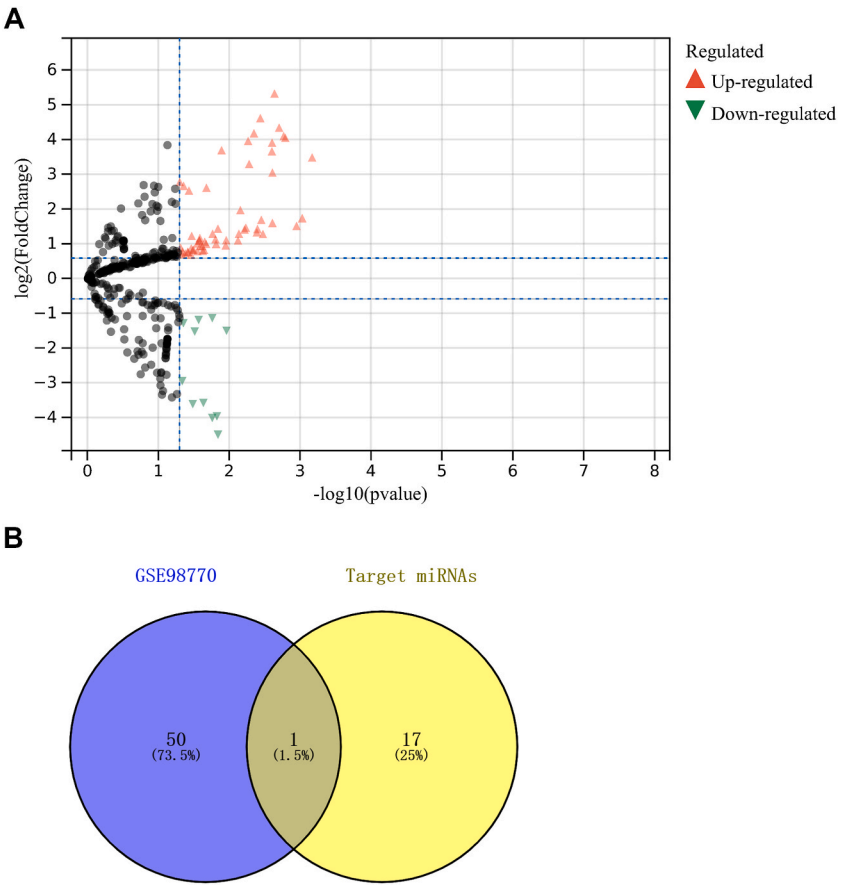


Fig. 5. Identification of the miRNAs regulating aging-related genes.
(A) Volcano diagram of GSE98770 dataset. (B) Venn diagram of differentially-expressed miRNAs of GSE98770 dataset and the predicted miRNAs of hub genes.

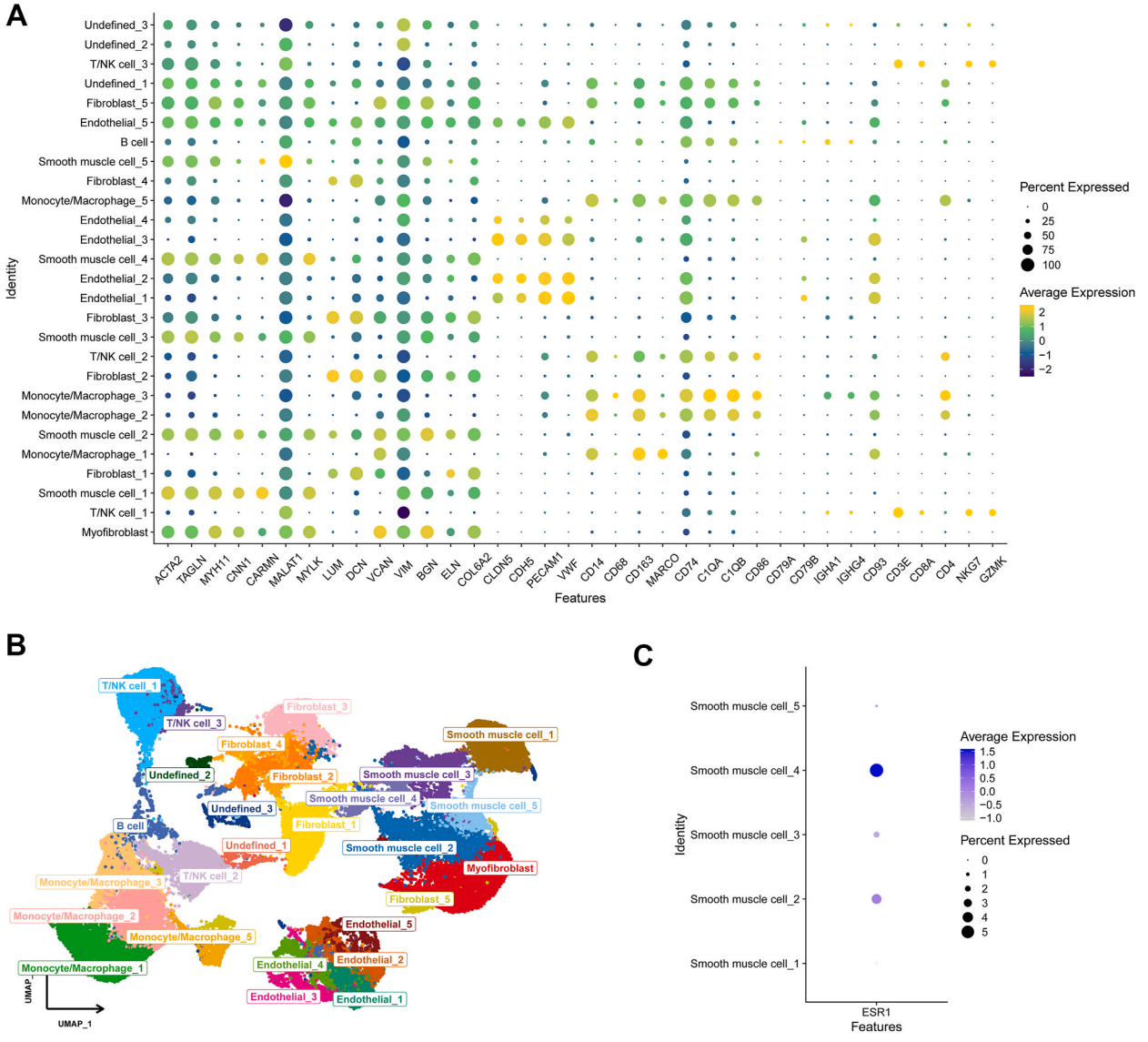


Fig. 6. The expression pattern of *ESR1* in SMC subpopulation in AD.
(A). Dot plot of the marker genes in distinct cell clusters of online scRNA-seq data. (B). Visualization of cell clusters that were identified in online scRNA-seq data was mapped using a tSNE map. (C). Dot plot of *ESR1* expression among distinct SMC subpopulations of online scRNA-seq data. (D). The dot plot revealed the expression of *ESR1* in SMC4 of AD and normal aortic tissues based on online scRNA-seq data. (E). UAMP plot showing cell clusters in our scRNA-seq data. (F). Dot plot showing the expression level of the *ESR1* gene in inter-SMC subclusters based on our scRNA-seq data. (G). Dot plot showing the difference in the expression level of *ESR1* in SMC3 between AD and normal aortic tissues in our scRNA-seq data.

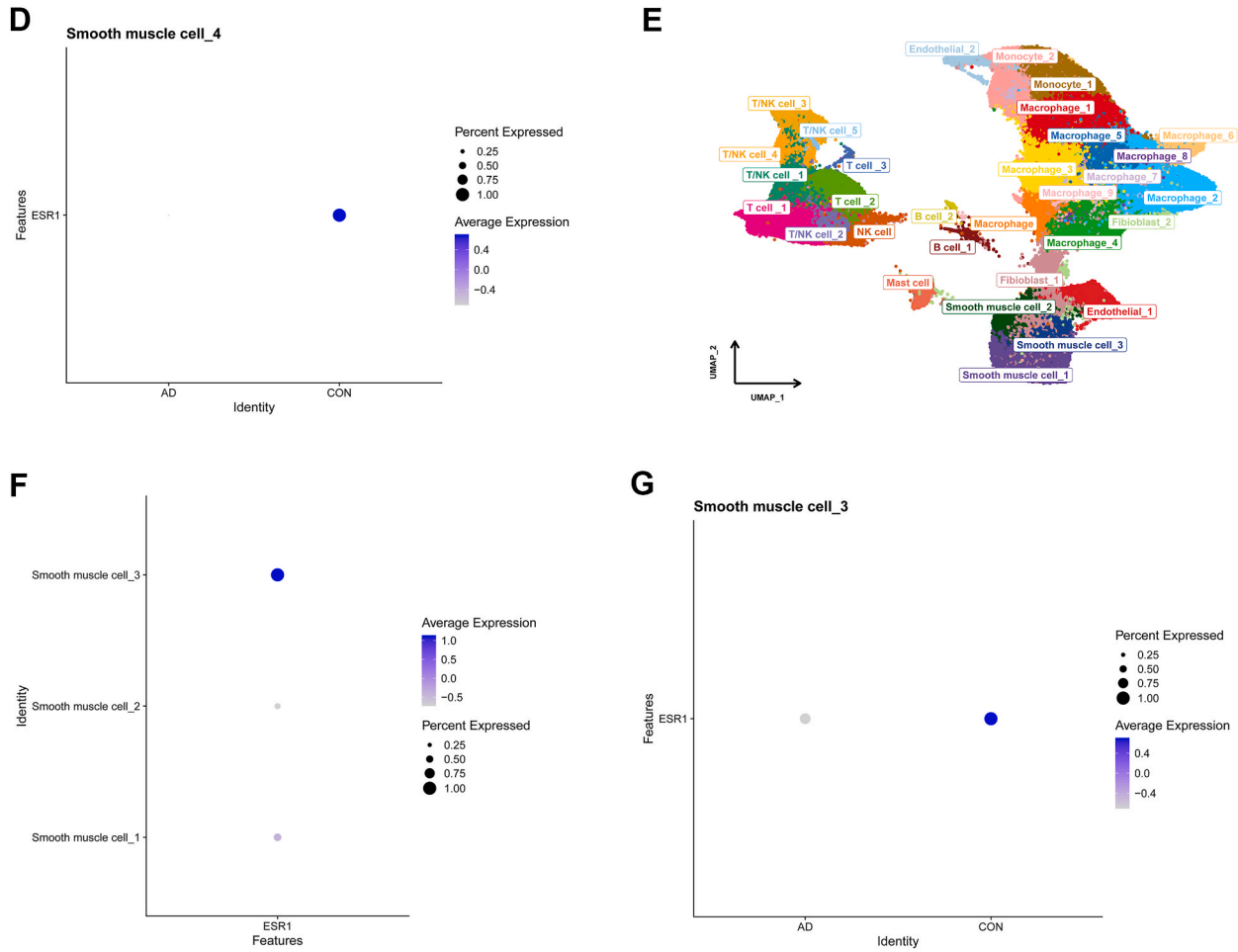


Fig. 6. (*continued*).

3.4. Functional enrichment analysis of hub genes

Functional enrichment analysis showed that hub genes were significantly enriched in the following terms, including "HIF-1 signaling pathway", "regulation of inflammation response" and "chemotaxis" ($P < 0.05$, Fig. 4).

3.5. miRNA-mRNA regulatory network construction

A total of 52 DEMis were identified in the GSE98770 dataset (Fig. 5A). Thirty-five miRNAs targeting hub genes were identified, among them, miR-221-3p was the only one in the intersection between DEMis and the predicted miRNAs targeting hub Args (Fig. 5B).

3.6. Cell expression pattern of ESR1 in SMCs in AD

Based on the online scRNA-seq data, a total of 5 smooth muscle cell (SMC) clusters were identified (Fig. 6A and 6B), including SMC1, SMC2, SMC3, SMC4 and SMC5. Differential gene expression analysis revealed that *ESR1* was primarily expressed by SMC4 (Fig. 6C). Further analysis showed that the expression of *ESR1* was lower in SMC4 in AD tissues compared to normal aortic tissues (Fig. 6D). Consistent with the results of online scRNA-seq data, our data also identified the presence of a special SMC cluster by sequencing 197,605 aortic cells, which manifested as reduced *ESR1* expression in AD tissues versus normal aortic dissection (Fig. 6E–6G). The upregulated genes of SMC4 were mainly related to "cellular senescence" and "cell cycle" (Fig. 7A and 7B), whereas the downregulated genes were mainly enriched in the "Apoptosis" pathway (Fig. 7C and 7D).

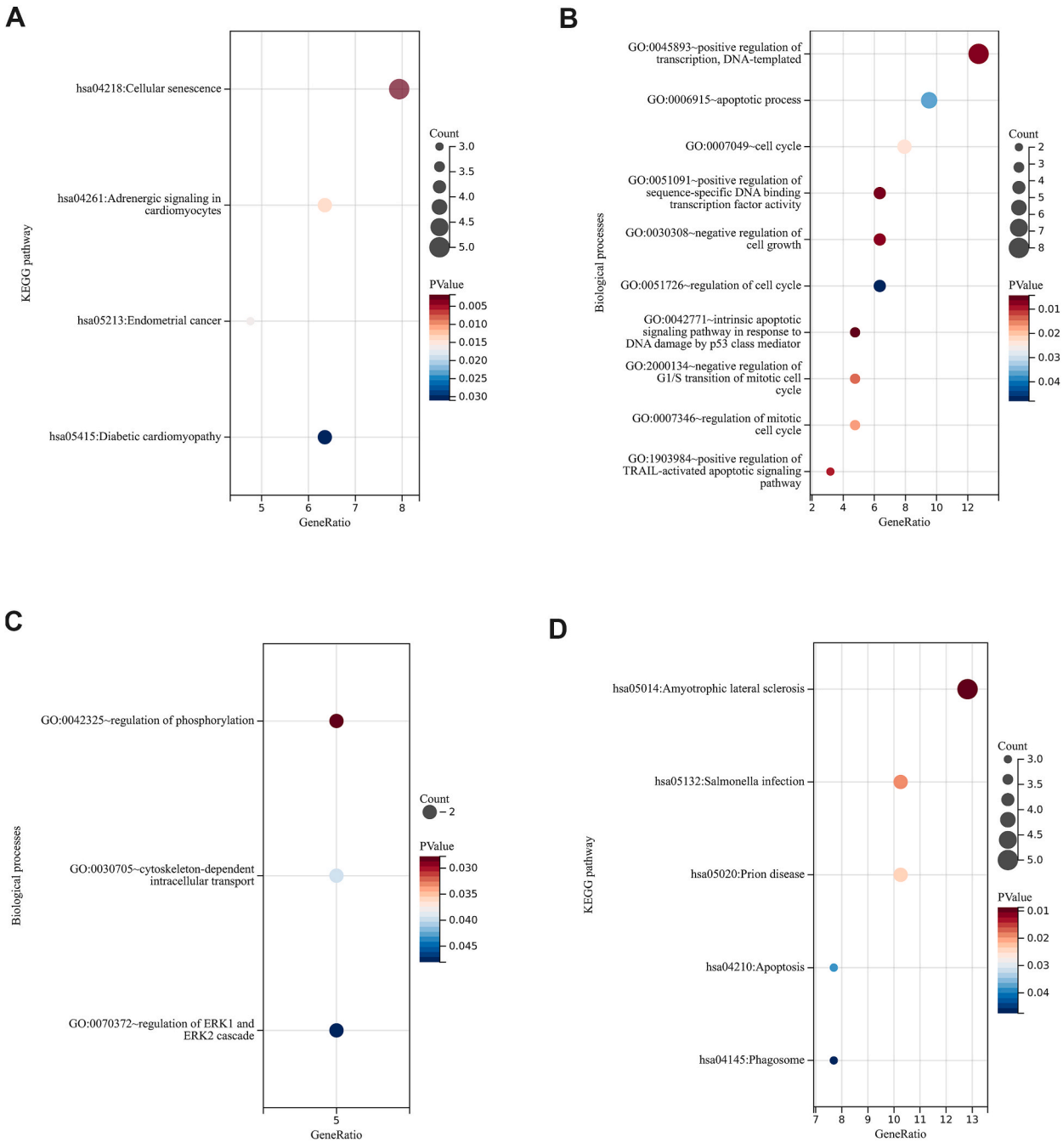


Fig. 7. Functional analysis of SMC4 in AD.
(A), (B) GO and KEGG enrichment analysis of upregulated genes. (C), (D) GO and KEGG enrichment analysis of downregulated genes.

Table 5
The characteristics of 11 candidate drugs targeting hub genes.

Drug	Genes	Interaction	Score	Drug Class
Imatinib mesylate	*HGF*	N/A	1.03	Antineoplastic agents, antineoplastic and immunomodulating agents, myelosuppressive agents
Talc	*CXCL8*	N/A	2.21	Not available
Cidofovir	*CXCL8*	N/A	1.1	Anti-infective agents, antiviral agents, nephrotoxic agents
Urokinase	*SERPINE1*	Substrate, inducer	3.09	Thrombolytic agents
Cetrorelix	*SERPINE1*	N/A	2.06	Fertility agents
Quinapril	*SERPINE1*	N/A	1.03	Antihypertensive agents
Clomiphene	*ESR1*	Antagonist, agonist	1.27	Fertility agents
Levonorgestrel	*ESR1*	Other	1.06	Contraceptive agents
Urokinase	*PLAUR*	Inducer, modulator	24.04	Thrombolytic agents
Filgrastim	*PLAUR*	N/A	4.12	Antineoplastic agents, immunomodulating agents
Ruxolitinib	*PLAUR*	N/A	2.29	Kinase inhibitors

Note: All drugs listed in the table have been approved by the US Food and Drug Administration.

3.7. Prediction of potential drugs of hub genes

A total of 11 existing drugs targeting 5 hub genes (*HGF*, *CXCL8*, *SERPINE1*, *ESR1*, *PLAUR*) were identified, among them, urokinase and quinapril have been reported to be associated with AD (Table 5).

4. Discussion

Several important findings were identified in this study, including 1) *HGF*, *CXCL8*, *SERPINE1*, *HIF1A*, *TIMP1*, *ESR1* and *PLAUR* were aging-related hub genes, which were enriched in terms of "HIF1-signaling", "regulation of inflammatory response" and "chemotaxis". 2) miR-221-3p was predicted to regulate *ESR1* expression. 3) A decreased *ESR1* expression was observed in SMC4 in AD tissues than that in normal aortic tissues, which was validated using our data. Furthermore, the "cellular senescence" pathway was significantly upregulated in SMC4 in AD versus normal aortic tissues. 4) Eleven existing drugs targeting hub Args were identified.

4.1. Aging-related hub genes and crucial pathway in AD

Among these 7 hub genes, *HGF*, *TIMP1*, *PLAUR*, *CXCL8*, *SERPINE1* and *HIF1A* were found to be upregulated in AD, while *ESR1* was downregulated. *HGF* encodes hepatocyte growth factor protein and plays an important role in regulating various biological processes, including alleviating inflammation, and tissue repair. A previous bioinformatic study identifies *HGF* as the hub gene of AD and further refers to *HGF* as a crucial immune-related gene [7]. In this study, *HGF* was identified as a crucial aging-related gene, which supports its pathogenic role in the development of AD. *HGF* is a hallmark of senescent fibroblasts, and the accumulation of senescent fibroblasts increases the risk of aging-related cardiac diseases by promoting fibrosis [8–10]. Furthermore, prior study reports that fibroblasts can contribute to AD formation by promoting excessive fibrosis [11]. These data indicates that *HGF* may promote fibroblast senescence to trigger fibrosis, which in turn leads to AD. Further studies are required to confirm this hypothesis. *CXCL8,* also known as *IL8*, is a common inflammatory cytokine and is positively associated with aging [12]. Consistent with previous studies [13], our study also identified increased *CXCL8* expression in AD. Overactivation of immune system inflammation is an important pathogenic characteristic of AD. Aging-induced inflammation contributes to aging diseases, indicating that *CXCL8* may serve as an important inflammatory factor related to aging-induced inflammation in the pathogenesis of AD. *SERPINE1*, which encodes the PAI-1 protein, is a hallmark of aging and cellular senescence [14,15]. In line with our study, a previous study reportes an increased *SERPINE1* in AD tissues [16], indicating that upregulated *SERPINE1* may be involved in AD formation as a crucial molecule for aging. The Urokinase plasminogen activator receptor, encoded by *PLAUR*, is a marker of chronic inflammation and can exacerbate aging [17]. Elevated *PLAUR* promotes vascular remodeling to promote chronic obstructive pulmonary disease development by promoting inflammation and extracellular matrix remodeling, which are important mechanisms for AD formation [18].*TIMP1* encodes tumor-derived protein tissue inhibitor of metalloproteinases-1 and serves as a regulator of the extracellular matrix. Compared to wild mice, a higher level of *TIMP1* is observed in the aortic tissues of the AD mouse model [19], supporting that *TIMP1* may be involved in the development of AD and further studies are required to investigate the role of *TIMP1* in the correlation between aging and AD. The pathogenic role of increased *HIF1A* in AD formation has been reported in several previous studies, including promoting inflammation, regulating SMC phenotype switch, and affecting SMC death [20,21]. Estrogen receptor-alpha (ERα), encoded by *ESR1*, mainly mediates the effects of estrogen, especially the main circulating estrogen 17β-estradiol [22]. *ESR1* is downregulated in aging arteries including the aorta, and deletion of *ESR1* reduces endothelium-dependent relaxation in the aorta [23–25], supporting the hypothesis that estrogen can prevent vascular aging. Nuclear activation of ERα alleviates arterial alternations in aging and hypertension mice [24], and early inactivation of membrane ERα contributes to endothelial dysfunction in aging mouse arteries [26]. These data indicates that activation of Erα may protect from vascular aging. Aging aorta undergoes remodeling, with collagen accumulation, reduced elastin fibres, advanced proinflammatory cytokines, and increased intimal thickening, resulting in elevated systolic blood pressure that increases the risk of aortic aneurysm and AD [27,28]. A previous study reportes a decreased *ESR1* level in aortic dilatation and AD compared to healthy

individuals [29]. Therefore, decreased *ESR1* may be involved in AD formation by promoting aorta aging. Consistent with this study, our data also identified a decreased *ESR1* in AD tissues, which further supports the potential pathogenic role of decreased *ESR1* in the development of AD KEGG enrichment analysis revealed that these hub genes were significantly enriched in the HIF-1 signaling pathway. HIF-1 signaling pathway, a crucial pathway for aging and aging-related diseases, cross-talks with various aging-related proteins, including sirtuins, *AMPK* and *NF-κB* [30,31]. In addition to the upregulated HIF-1 signaling pathway, sirtuins, *AMPK*, and *NF-κB* are also associated with AD formation [32], indicating that the HIF-1 signaling pathway may be the key pathway regulating aging in the pathogenesis of AD.

4.2. ESR1 of SMCs in AD

In this study, both online and our single-cell RNA sequencing data showed a reduction in *ESR1* in AD tissues compared to normal aortic tissues. In addition, the upregulated genes in SMC4 in AD tissues were mainly enriched in "cellular senescence". In line with our results, previous studies find that the mRNA and protein expression levels of *ESR1* are decreased in senescent SMCs compared to young SMCs [33,34]. Estrogen protects against SMC premature senescence, and inhibition of Erα significantly suppressed the senescent-inhibiting effects of estrogen [35], indicating that decreased *ESR1* may promote SMC senescence. A previous study reports that senescent SMCs contribute to inflammation and degradation of the extracellular matrix, and inhibition of SMC senescence has been found to reduce the information of experimental AD [36]. Therefore, we supposed that *ESR1* may be involved in AD formation, and SMC senescence was the underlying mechanism. Replicative senescence is the first subtype of senescent cell to be described, and it is characterized by telomere shorting and dysfunctional telomeres [37]. Telomere maintenance and telomere attrition-associated senescence of endothelial and SMCs have been indicated to be part of the pathogenesis of degenerative vascular diseases [38]. Shorten telomere length is associated with an increased risk of AD [39]. However, genes involved in the negative regulation of cell proliferation were upregulated in SMC4 in AD tissues versus normal aortic tissues, indicating that *ESR1* may not regulate SMC4 aging via regulating replicative senescence. *cGAS-STING (a DNA damage-related signaling)* activation occurs in late senescence [40]. Activation of the *cGAS-STING* axis promotes AD formation by promoting SMC apoptosis and macrophage recruitments by *STING-IRF3* activation [41], indicating that the *cGAS-STING* axis may play an important role in SMC4 senescence in the development of AD, which should be confirmed by further study. In addition, senescence-associated secretory phenotype (SASP) is a common feature of senescent cells, characterized by increased secretion of pro-inflammatory cytokines and matrix-remodeling enzymes [42,43], while both inflammation and extracellular matrix remodeling are the crucial mechanisms for AD [44,45], indicating that SPAP may be another potential mechanism for aging SMC contributes to AD. In summary, the *cGAS-STING-IRF3* axis and SPAP may be the potential mechanisms for SMC4 aging increasing the risk of AD, which needs to be confirmed in a future study.

4.3. miRNA-mRNA regulatory network

miR-221-3p was predicted to regulate the expression of *ESR1* in this study. miR-221-3p plays an important role in pro-fibrosis, anti-inflammation and anti-angiogenesis [46,47]. Compared to young spontaneously hypertensive rats, rno-miR-221-3p was significantly upregulated in aging spontaneously hypertensive rats [48]. Additionally, inhibition of miR-221-3p protects against doxorubicin-induced cardiac senescence [49]. Therefore, we hypothesized that miR-221-3p may be a crucial miRNA involved in cardiovascular aging, and more study is required to confirm our hypothesis.

4.4. Potential drugs targeting hub genes

Among 11 predicted drugs, ruxolitinib and filgrastim have been reported to be associated with AD. Ruxolitinib, a kinase inhibitor, reduces the prevalence of AD by inhibiting *JAK2* expression [50]. Our results identified ruxolitinib as the potential target drug for *PLAUR*, indicating that administration of ruxolitinib may alleviate AD formation by inhibiting aortic aging. Filgrastim is a recombinant human granulocyte-colony stimulating factor. Interestingly, filgrastim increases rather than decreases the risk of AD [51–53]. In this study, *PLAUR* was also identified as the targeted gene of filgrastim, supporting a crucial role for *PLAUR* in AD formation. Further study is required to explore the function of *PLAUR* in the processes that filgrastim contributes to AD. Currently, filgrastim is widely applied in tumor treatment and organ transplant [54]. Therefore, we supposed that patients with tumors should use filgrastim with caution, and the best optional therapeutic dose for filgrastim is needed to be determined in further studies. Urokinase also known as uPA, plays an important role in vascular remodeling. Overexpression of uPA contributes to experimental AAA by promoting ECM degradation via activating MMPs [55]. Administration of uPA may also be a trigger of AD, for the similarity of pathological mechanism between AD and AAA. The associations between the remaining predictive drugs (imatinib mesylate, talc, cidofovir, cetrorelix, quinapril, clomiphene, levonorgestrel) and AD remain elusive.

5. Limitations

The main limitation of our study is the lack of validation in vitro experiments. Unavoidable biases, such as differences in gender and age are another notable limitation. Thus, the next step of our study is to analyze the expression of *ESR1* protein and aging-canonical markers in SMCs present in aortic dissection tissues, if the results are consistent with our findings, we will further investigate how *ESR1* contribute to SMC aging to induce AD using *ESR1* knockout and overexpression SMCs and mice.

6. Conclusion

This study identified several important findings of the aging mechanisms underlying AD: *HGF*, *CXCL8*, *SERPINE1*, *HIF1A*, *TIMP1*, *ESR1* and *PLAUR* were the aging-related hub genes involved in AD development, and the HIF-1 singling pathway was the key pathway regulating aging. miR-221-3p was predicted to regulate the expression of *ESR1*. In addition, this study proposed a hypothesis that the downregulation of *ESR1* drives SMC aging in the pathogenesis of AD, which requires further experiments to confirm.

Declarations ethics approval and consent to participate

This study was approved by the Ethics Committee of The First Affiliated Hospital of Shantou University Medical College (No. B-2021-114). All patients provided informed consent before they participated in the study.

Data availability statement

The single-cell RNA sequencing data of 13 AD patients is available from the corresponding author upon reasonable request, and the rest transcriptomic data of AD analyzed in this study are available from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database.

Funding

This study was supported by the Science and Technology Special Fund of Guangdong Province in 2021 ("Big task" and "Task list") (No.20220616017-2).

CRediT authorship contribution statement

Jingyi Yan: Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Xuerui Tan:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Dr. Stanley Li Lin, Department of Cell Biology and Genetics, Shantou University Medical College, for his English language polish.

References

[1] K.M. Harris, et al., Early mortality in type A acute aortic dissection: insights from the international registry of acute aortic dissection, JAMA Cardiol 7 (10) (2022) 1009–1015.
[2] J. Golledge, K.A. Eagle, Acute aortic dissection, Lancet 372 (9632) (2008) 55–66.
[3] E. Bossone, K.A. Eagle, Epidemiology and management of aortic disease: aortic aneurysms and acute aortic syndromes, Nat. Rev. Cardiol. 18 (5) (2021) 331–348.
[4] M. Landenhed, et al., Risk profiles for aortic dissection and ruptured or surgically treated aneurysms: a prospective cohort study, J. Am. Heart Assoc. 4 (1) (2015) e001513.
[5] D.P. Howard, et al., Population-based study of incidence and outcome of acute aortic dissection and premorbid risk factor control: 10-year results from the Oxford Vascular Study, Circulation 127 (20) (2013) 2031–2037.
[6] T.J. Schlatmann, A.E. Becker, Pathogenesis of dissecting aneurysm of aorta. Comparative histopathologic study of significance of medial changes, Am. J. Cardiol. 39 (1) (1977) 21–26.
[7] F. Chen, J. Han, B. Tang, Patterns of immune infiltration and the key immune-related genes in acute type A aortic dissection in bioinformatics analyses, Int. J. Gen. Med. 14 (2021) 2857–2869.
[8] Z. Qin, C.A. Worthen, T. Quan, Cell-size-dependent upregulation of HGF expression in dermal fibroblasts: impact on human skin connective tissue aging, J. Dermatol. Sci. 88 (3) (2017) 289–297.
[9] R. Vidal, et al., Transcriptional heterogeneity of fibroblasts is a hallmark of the aging heart, JCI Insight 4 (22) (2019).
[10] F. Zhu, et al., Senescent cardiac fibroblast is critical for cardiac fibrosis after myocardial infarction, PLoS One 8 (9) (2013) e74535.
[11] Y. Chen, et al., Dysregulation of interaction between LOX(high) fibroblast and smooth muscle cells contributes to the pathogenesis of aortic dissection, Theranostics 12 (2) (2022) 910–928.
[12] H.L. Bonfante, et al., CCL2, CXCL8, CXCL9 and CXCL10 serum levels increase with age but are not altered by treatment with hydroxychloroquine in patients with osteoarthritis of the knees, Int J Rheum Dis 20 (12) (2017) 1958–1964.
[13] C. Doppler, et al., Immune response associated gene signatures in aortic dissection compared to aortic aneurysm, Front Biosci (Landmark Ed) 29 (2) (2024) 64.
[14] D.E. Vaughan, et al., Plasminogen activator inhibitor-1 is a marker and a mediator of senescence, Arterioscler. Thromb. Vasc. Biol. 37 (8) (2017) 1446–1452.
[15] S.S. Khan, et al., A null mutation in SERPINE1 protects against biological aging in humans, Sci. Adv. 3 (11) (2017) eaao1617.
[16] D. Li, et al., SERPINE1 gene is a reliable molecular marker for the early diagnosis of aortic dissection, Evid Based Complement Alternat Med 2022 (2022) 5433868.

[17] L.J.H. Rasmussen, et al., Association between elevated suPAR, a new biomarker of inflammation, and accelerated aging, J Gerontol A Biol Sci Med Sci 76 (2) (2021) 318–327.
[18] K. Egervall, A. Rosso, S. Elmståhl, Association between cardiovascular disease- and inflammation-related serum biomarkers and poor lung function in elderly, Clin. Proteonomics 18 (1) (2021) 23.
[19] J. Xiong, et al., Downregulation of LILRB4 promotes human aortic smooth muscle cell contractile phenotypic switch and apoptosis in aortic dissection, Cardiovasc. Toxicol. 24 (3) (2024) 225–239.
[20] B. Huang, et al., HIF-1α contributes to hypoxia-induced VSMC proliferation and migration by regulating autophagy in type A aortic dissection, Adv Biol (Weinh) 8 (1) (2024) e2300292.
[21] G. Lian, et al., Macrophage metabolic reprogramming aggravates aortic dissection through the HIF1α-ADAM17 pathway(☆), EBioMedicine 49 (2019) 291–304.
[22] J.F. Arnal, et al., Estrogen receptors and endothelium, Arterioscler. Thromb. Vasc. Biol. 30 (8) (2010) 1506–1512.
[23] K.M. Gavin, et al., Vascular endothelial estrogen receptor alpha is modulated by estrogen status and related to endothelial function and endothelial nitric oxide synthase in healthy women, J. Clin. Endocrinol. Metab. 94 (9) (2009) 3513–3520.
[24] E. Guivarc'h, et al., Nuclear activation function 2 estrogen receptor α attenuates arterial and renal alterations due to aging and hypertension in female mice, J. Am. Heart Assoc. 9 (5) (2020) e013895.
[25] R. Gurrala, et al., Alterations in the estrogen receptor profile of cardiovascular tissues during aging, Geroscience 43 (1) (2021) 433–442.
[26] J. Favre, et al., Early inactivation of membrane estrogen receptor alpha (ERα) recapitulates the endothelial dysfunction of aged mouse resistance arteries, Int. J. Mol. Sci. 23 (5) (2022).
[27] J.A. Collins, et al., The anatomy of the aging aorta, Clin. Anat. 27 (3) (2014) 463–466.
[28] G.L. Pierce, et al., Is it good to have a stiff aorta with aging? Causes and consequences, Physiology 37 (3) (2022) 154–173.
[29] Y. Zhang, et al., Low expression of ESR1 correlates with ascending aortic dilation and acute type A aortic dissection, Gene 851 (2022) 147001.
[30] A.A. van Beek, et al., Metabolic alterations in aging macrophages: ingredients for inflammaging? Trends Immunol. 40 (2) (2019) 113–127.
[31] E.J. Yeo, Hypoxia and aging, Exp. Mol. Med. 51 (6) (2019) 1–15.
[32] R. Lian, et al., Identification of molecular regulatory features and markers for acute type A aortic dissection, Comput. Math. Methods Med. 2021 (2021) 6697848.
[33] H. Wan, et al., Bioinformatics analysis of aging-related genes in thoracic aortic aneurysm and dissection, Front Cardiovasc Med 10 (2023) 1089312.
[34] F. Huang, et al., GPR30 decreases with vascular aging and promotes vascular smooth muscle cells maintaining differentiated phenotype and suppressing migration via activation of ERK1/2, OncoTargets Ther. 9 (2016) 3415–3422.
[35] C. Zhu, et al., Effects of estrogen on stress-induced premature senescence of vascular smooth muscle cells: a novel mechanism for the "time window theory" of menopausal hormone therapy, Atherosclerosis 215 (2) (2011) 294–300.
[36] W.M. Zhang, et al., Sustained activation of ADP/P2ry12 signaling induces SMC senescence contributing to thoracic aortic aneurysm/dissection, J. Mol. Cell. Cardiol. 99 (2016) 76–86.
[37] L. Hayflick, P.S. Moorhead, The serial cultivation of human diploid cell strains, Exp. Cell Res. 25 (1961) 585–621.
[38] T. Aschacher, et al., Telomere Biology and thoracic aortic aneurysm, Int. J. Mol. Sci. 19 (1) (2017).
[39] J. Yan, et al., Short leukocyte telomere length is associated with aortic dissection, Intern. Med. 50 (23) (2011) 2871–2875.
[40] L. Roger, F. Tomas, V. Gire, Mechanisms and regulation of cellular senescence, Int. J. Mol. Sci. 22 (23) (2021).
[41] W. Luo, et al., Critical role of cytosolic DNA and its sensing adaptor STING in aortic degeneration, dissection, and rupture, Circulation 141 (1) (2020) 42–66.
[42] J.P. Coppé, et al., Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor, PLoS Biol. 6 (12) (2008) 2853–2868.
[43] C.D. Wiley, et al., SILAC analysis reveals increased secretion of hemostasis-related factors by senescent cells, Cell Rep. 28 (13) (2019) 3329–3337.e5.
[44] L. Han, et al., CD40L promotes development of acute aortic dissection via induction of inflammation and impairment of endothelial cell function, Aging (Albany NY) 10 (3) (2018) 371–385.
[45] B.O. Aicher, et al., Moderate aerobic exercise prevents matrix degradation and death in a mouse model of aortic dissection and aneurysm, Am. J. Physiol. Heart Circ. Physiol. 320 (5) (2021) H1786–h1801.
[46] Z.Q. Jin, MicroRNA targets and biomarker validation for diabetes-associated cardiac fibrosis, Pharmacol. Res. 174 (2021) 105941.
[47] Y. Li, et al., MiR-221-3p targets Hif-1α to inhibit angiogenesis in heart failure, Lab. Invest. 101 (1) (2021) 104–115.
[48] J. Wang, et al., Differential microRNA expression profiles and bioinformatics analysis between young and aging spontaneously hypertensive rats, Int. J. Mol. Med. 41 (3) (2018) 1584–1594.
[49] L. Zhuang, et al., Exosomal LncRNA-NEAT1 derived from MIF-treated mesenchymal stem cells protected against doxorubicin-induced cardiac senescence through sponging miR-221-3p, J. Nanobiotechnol. 18 (1) (2020) 157.
[50] R. Al-Rifai, et al., JAK2V617F mutation drives vascular resident macrophages toward a pathogenic phenotype and promotes dissecting aortic aneurysm, Nat. Commun. 13 (1) (2022) 6592.
[51] J. Nakamura, et al., Pegfilgrastim-associated large-vessel vasculitis developed during adjuvant chemotherapy for breast cancer: a case report and review of the literature, J. Oncol. Pharm. Pract. 26 (7) (2020) 1785–1790.
[52] Y. Sato, et al., Thoracic aortitis and aortic dissection following pegfilgrastim administration, Eur. J. Cardio. Thorac. Surg. 52 (5) (2017) 993–994.
[53] E. Shiraki, et al., Aortitis and aortic dissection after administration of pegfilgrastim during adjuvant chemotherapy for early breast cancer, Int Cancer Conf J 11 (2) (2022) 138–141.
[54] P. Cornes, A. Krendyukov, The evolution of value with filgrastim in oncology, Future Oncol. 15 (13) (2019) 1525–1533.
[55] P. Carmeliet, et al., Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation, Nat. Genet. 17 (4) (1997) 439–444.