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Original Article

Identification of potential biomarkers associated with aging through *in silico* analysis

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ABSTRACT

Objectives: In this study, we aim to investigate transcriptomic and immune alterations associated with chronological aging by analyzing the GSE237029 dataset, which includes peripheral blood mononuclear cell (PBMC) gene expression profiles from middle-aged and elderly individuals.

Materials and methods: We analyzed the publicly available GSE237029 dataset comparing gene expression profiles in PBMCs between two age groups [n=4 middle-aged (35-50 years), n=5 older adults (75-89 years)]. We identified differentially expressed genes (DEGs), assessed their functional significance through pathway enrichment analyses, examined differences in immune cell composition, and investigated shared micro-ribonucleic acid (miRNA) and transcription factor (TF) regulators. Functional enrichment analyses were performed using g:Profiler. Immune cell composition differences were assessed via ImmuCellAI, and shared miRNA and TF regulators of DEGs were identified using miRDIP and TRRUST databases.

Results: A total of 19 DEGs were identified, showing enrichment in pathways related to phosphatidylinositol 3-kinase signaling, signal transducer and activator of transcription (STAT) phosphorylation regulation, ubiquitin-mediated proteolysis, and cytokine-driven immune responses. Immune cell infiltration analysis revealed notable differences in induced regulatory T-cells, central memory T-cells, and effector memory T-cells across different age groups. Regulatory network analysis identified ten candidate miRNAs and six TFs as key upstream regulators of age-related DEGs. Notably, TEA domain transcription factor 4 within the Hippo-YAP/TAZ-TEAD pathway was recognized as a central TF targeting multiple aging-associated miRNAs, suggesting its potential role in influencing senescence and immune remodeling.

Conclusion: Our *in silico* analysis identifies a set of PBMC-derived molecular signatures, including nuclear factor kappa-light-chain-enhancer of activated B cells/STAT-associated DEGs, specific miRNAs, and TF-miRNA regulatory axes that could serve as promising minimally invasive biomarkers for aging. These findings support combining transcriptomic and regulatory network profiling to better understand immune aging and guide targeted anti-aging strategies.

Keywords: Aging, biomarkers, immune cell infiltration, *in silico* analysis, miRNA.

Aging is a complex biological process characterized by a gradual decline in physiological functions and increased susceptibility to various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders.[1] Previous research indicated that aging is driven by interconnected biological hallmarks, including genomic instability, telomere attrition, mitochondrial dysfunction, stem cell exhaustion, and, notably, altered intercellular communication.[2] These molecular changes gradually impair tissue homeostasis and create a pro-inflammatory environment that particularly increases the risk of immune dysregulation in aging organisms.

A key feature of aging is the disruption of intercellular communication and changes in immune system behavior.[3] Age-related immune system changes, known as inflammaging, lead to chronic, low-grade inflammation and weaken adaptive immune responses, thereby increasing vulnerability to disease and death.[4] Aging also significantly impacts innate immunity; neutrophils in older adults show altered functions, including increased pro-inflammatory activity

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such as heightened neutrophil extracellular trap formation, dysregulated tissue infiltration, and reduced efficiency in clearing pathogens.[5,6] Additionally, elderly individuals show a significant decline in neutrophil effector functions, including impaired migration, decreased phagocytic capacity, and lower cluster of differentiation (CD) 16 expression.[7,8] These changes in adaptive and innate immunity increase the risk of infections, cancer, and chronic inflammatory conditions associated with aging.

Recent advances in transcriptomic technologies have enabled investigating gene expression changes related to aging across different tissues. Analyzing gene expression changes in peripheral blood mononuclear cells (PBMCs) is advantageous as they can be accessed through a non-invasive approach and provide a dynamic representation of the immune system's status in circulation.[9] Although several studies have identified distinct molecular signatures of aging in blood, such as circulating micro-ribonucleic acids (miRNAs) like miR-181a and miR-1248, large-scale PBMC-specific miRNA or transcription factor (TF) signatures are still not well defined.[10] A recent longitudinal single-cell transcriptomic study observed age-related changes in immune cell composition, specifically a decrease in naïve $CD8^+$ T-cells and an increase in non-classical monocytes. However, the role of miRNAs or TF regulatory networks contributing to the aging process has not been clearly presented.[11] Furthermore, the development of cell-type-specific aging clocks, such as the sc-ImmuAging model that applies single-cell aging trajectories to monocytes, T-cells, and natural killer (NK) cells, provides a promising framework for future research, despite the upstream molecular regulators being unknown.[12] While early aging patterns are beginning to be identified, comprehensive molecular signatures of PBMCs remain limited. This highlights the urgent need for integrated multi-omics and mechanistic validation studies.

In this study, we aimed to explore the transcriptomic and immune changes associated with chronological aging by analyzing the GSE237029 dataset, which compares the gene expression profiles of PBMC from middle-aged and older adults. Our study presents a comprehensive molecular profile of aging by *in silico* approaches and identifies key regulatory biomarkers critical for understanding age-related dysfunction.

MATERIALS AND METHODS

Data retrieval and analysis of differentially expressed genes

The NCBI Gene Expression Omnibus (GEO) was used to identify publicly available gene expression datasets for examining age-related transcriptomic changes. The GSE237029 dataset, which includes a peripheral blood RNA profile generated by Affymetrix Human Genome U133 Plus 2.0 Array, was filtered by considering the specific keywords: PBMC, old age, gene expression, and microarray. The selected dataset includes nine samples from two age-defined groups: middle-aged individuals (35-50 years) and older adults (75-89 years). Geo2R was used to perform differential expression analysis by implementing the limma package in R, with a log fold change (FC) >1 or logFC <-1 and a significance threshold of $p<0.05$. The 'pheatmap' and 'ggplot2' packages were used to create heatmaps and volcano plots, respectively. Venn diagrams were employed to identify and visualize differentially expressed genes (DEGs) between groups. Functional enrichments of the DEGs were determined using the g:Profiler.[13]

Immune cell infiltration analysis

We determined the distribution of immune cell composition between age groups using the ImmuCellAI web tool. Raw data of GSE237029 was uploaded to the ImmuCellAI tool, and the abundance of 18 T-cell subtypes and six other immune cells (B cells, NK cells, monocytes, macrophages, neutrophils, and dendritic cells) were estimated by comparing to the reference dataset.[14]

Identification of common micro-ribonucleic acids and transcription factor regulators

The microRNA Data Integration Portal (https://ophid.utoronto.ca/mirDIP/index_confirm.jsp) was used to find shared miRNA regulators of the identified DEGs. The score class threshold was set to “high” to ensure the predictions were accurate.[15] The common TFs of DEGs were extracted using the TRRUST website.[16]

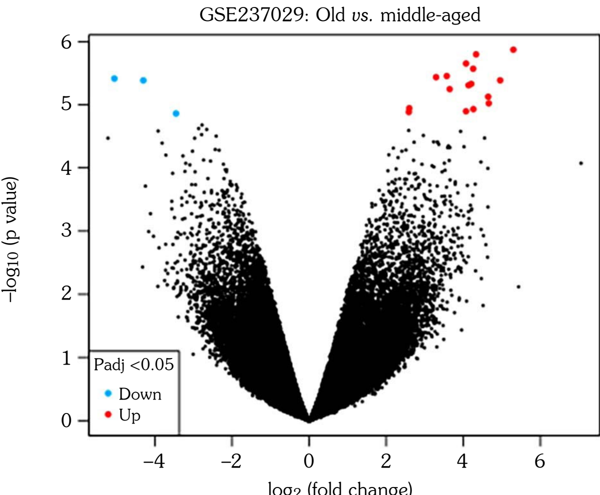

Figure 1. Volcano plot illustrating differential gene expression between older and middle-aged groups. Genes with statistically significant differential expression (Padj <0.05) are marked in red (upregulated) and blue (downregulated). The x-axis shows the $\log_2$ fold change, while the y-axis displays the $-\log_{10}$ (p-value).

RESULTS

Functional relevance of differentially expressed genes

Geo2R analysis revealed a total of 19 DEGs between middle-aged and older adults, of which 3 were downregulated and 16 were upregulated, as shown in Figure 1. Pathway enrichment analysis showed that the DEGs were significantly linked to various signaling and regulatory processes, as shown in Table 1. Several enriched terms associated with the phosphatidylinositol 3-kinase (PI3K) pathway, including molecular functions and cellular components, indicate a potential role for PI3K signaling in age-related gene expression changes. Additionally, terms related to signal transducer and activator of transcription (STAT) protein phosphorylation regulation, ubiquitin-mediated proteolysis, and cytokine signaling (such as interferon-gamma regulation) were prominent across GO (gene ontology): Biological pathway, KEGG (Kyoto Encyclopedia of Genes and Genomes), and Reactome databases. The enrichment of leptin/insulin and macrophage-stimulating protein pathways further supports the involvement of metabolic and immune-modulatory processes. These findings suggest the age-related DEGs are functionally enriched in pathways involved in signal transduction, protein degradation, and immune signaling, which may contribute to the physiological differences observed between the older and middle-aged groups.

Immune cell infiltration analysis

Immune cell infiltration analysis was conducted by examining the expression profiles of two groups (middle-aged individuals and older adults), which were evaluated using an reference expression matrix comprising 415 datasets derived from 26 studies that were manually curated. The distribution of immune cells in two groups is shown in Figure 2a, b. In the dataset, the distributions of induced regulatory T-cells (iTreg) (p=0.019), central memory T-cells (Tcm) (p=0.037), and effector memory T-cells (Tem) (p=0.044) were significant between the two groups.

Table 1. Functional pathway enrichment of differentially expressed genes

Category	Term name	Term ID	Adjusted p-value
GO: Molecular function	1-phosphatidylinositol-3-kinase regulator activity	GO: 0046935	1.847×10^{-2}
GO: Molecular function	1-phosphatidylinositol-3-kinase activity	GO: 0016303	4.598×10^{-2}
GO: Biological process	Negative regulation of tyrosine phosphorylation of STAT protein	GO: 0042532	2.706×10^{-2}
GO: Cellular component	Phosphatidylinositol 3-kinase complex	GO: 0005942	2.774×10^{-2}
GO: Cellular component	Extrinsic component of the membrane	GO: 0019898	4.526×10^{-2}
KEGG	Ubiquitin-mediated proteolysis	KEGG: 04120	1.857×10^{-2}
Reactome pathway	Regulation of IFNG signaling	R-HSA-877300	2.154×10^{-2}

GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; STAT: Signal transducer and activator of transcription.

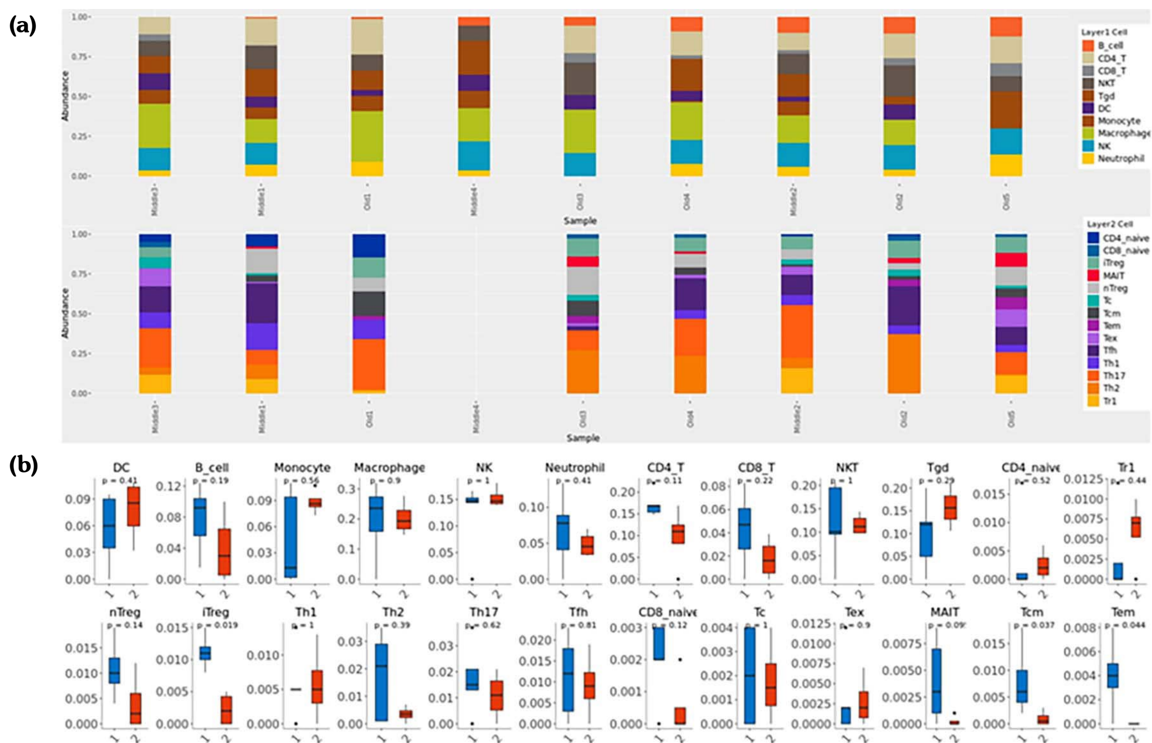

Figure 2. **(a)** Distributions of layer 1 and layer 2 immune cell types. **(b)** Comparison of immune cell distribution between groups.

Values exhibiting statistical significance are indicated with an asterisk.

Common regulators of shared differentially expressed genes

Our analysis revealed ten miRNAs (hsa-miR-223-3p, hsa-miR-561-3p, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-26b-5p, hsa-miR-3920, hsa-miR-3976, hsa-miR-5010-3p, hsa-miR-548x-3p, hsa-miR-603) targeting at least five DEGs, as shown in Table 2. Moreover, six TFs [v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA), nuclear factor kappa B subunit 1

Table 2. Common miRNA regulators of differentially expressed genes

miRNAs\gene	C1QC	DNAJC21	DSC2	ERAP2	MFSD6	NAMPT	PKNOX1	SDC2	SIAH1	SOCS1	SOCS3	TET3	Shared genes
hsa-miR-223-3p					Y	Y	Y	Y	Y			Y	6
hsa-miR-561-3p		Y			Y	Y		Y		Y	Y		6
hsa-miR-19a-3p					Y		Y			Y	Y	Y	5
hsa-miR-19b-3p					Y		Y			Y	Y	Y	5
hsa-miR-26b-5p		Y	Y		Y	Y						Y	5
hsa-miR-3920			Y		Y	Y		Y	Y				5
hsa-miR-3976		Y	Y					Y			Y	Y	5
hsa-miR-5010-3p		Y	Y		Y	Y						Y	5
hsa-miR-548x-3p		Y	Y			Y		Y	Y				5
hsa-miR-603		Y	Y				Y		Y			Y	5

miRNA: Micro-ribonucleic acids.

Table 3. Transcription factor regulators of common miRNA regulators of differentially expressed genes

Key TF	Number of overlapped genes	*p*	Q value	List of overlapped genes
RELA	4	4.05E-05	0.000125	ERAP2, CXCL8, NAMPT, SOCS3
NFKB1	4	4.15E-05	0.000125	SOCS3, CXCL8, NAMPT, ERAP2
STAT3	3	0.000113	0.000226	SOCS1, SOCS3, CXCL8
STAT6	2	0.000272	0.000409	CXCL8, SOCS1
IRF1	2	0.000548	0.000658	ERAP2, SOCS1
JUN	2	0.00456	0.00456	CXCL8, NAMPT

miRNA: Micro-ribonucleic acids; TF: Transcription factor.

Table 4. Common transcription factor regulators of miRNAs

Transcription factor	Count	Percent	Fold	*p*	Bonferroni	FDR	Common miRNAs
TLX1	3	0.076923	15.5	0.0009	0.551	0.551	miR-26b, miR-548aq, miR5696
TLX3	4	0.031008	6.248062	0.002739	1	0.8116	hsa-miR-5010, hsa-miR-19a, hsa-miR-223, hsa-miR-19b
MAPK14	2	0.111111	22.38889	0.003978	1	0.8116	hsa-miR-19a, hsa-miR-19b
TEAD4	7	0.010355	2.086538	0.020784	1	0.9834	hsa-miR-5010, hsa-miR-561, hsa-miR-19a, hsa-miR-26b, hsa-miR-603, hsa-miR-548x, hsa-miR-223
FOXC1	5	0.01269	2.557107	0.030527	1	0.9834	hsa-miR-5010, hsa-miR-26b, hsa-miR-223, hsa-miR-603, hsa-miR-19a
EN1	2	0.033333	6.716667	0.035436	1	0.9834	hsa-miR-26b, hsa-miR-603
SAFB	3	0.018987	3.825949	0.039038	1	0.9834	hsa-miR-561, hsa-miR-19a, hsa-miR-26b
NKX2-5	2	0.028571	5.757143	0.046648	1	0.9834	hsa-miR-19a, hsa-miR-19b

miRNA: Micro-ribonucleic acids; miRNAs: Micro-ribonucleic acids.

(NFKB1), STAT3, STAT6, interferon regulatory factor 1 (IRF1), Jun proto-oncogene, AP-1 transcription factor subunit (JUN)] were found to be regulating at least two DEGs, as shown in Table 3. We also examined the TF-miRNA interactions and found that eight [T-cell leukemia homeobox 1 (TLX1), T-cell leukemia homeobox 3 (TLX3), mitogen-activated protein kinase 14 (MAPK14), TEA domain transcription factor 4 (TEAD4), forkhead box C1 (FOXC1), EN1 (engrailed homeobox 1), scaffold attachment factor B (SAFB), NK2 homeobox 5 (NKX2-5)] TFs targeted at least two miRNAs, as shown in Table 4. The

TEAD4 is identified to regulate eight miRNAs (hsa-miR-5010, hsa-miR-561, hsa-miR-19a, hsa-miR-26b, hsa-miR-603, hsa-miR-548x, hsa-miR-223).

DISCUSSION

In this study, we explored distinct molecular signatures between two age groups (middle-aged *vs.* older adults) using public microarray data that may be utilized in aging-related interventions.

Our results show that immune responses are actively reshaped during aging, with miRNAs and TFs playing important regulatory roles. Among the miRNAs identified, hsa-miR-223-3p is well-known for its role in immune cell differentiation and inflammation regulation. Previously, altered expression of this miRNA has been linked to immune aging in older adults.[17] The miR-19 family (miR-19a/b) has also been shown to influence signaling pathways relevant to aging through PI3K/protein kinase B (AKT) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways.[18] Additionally, members of the miR-26 family are reported to be associated with senescence and cell cycle regulation by affecting the p53/p21 axis during senescence.[19,20] The fact that these miRNAs are predicted to target multiple DEGs in our dataset suggests coordinated post-transcriptional control mechanisms contributing to immune remodeling during aging.

Our analysis also identified key TFs (RELA, NFKB1, STAT3, STAT6, IRF1, and JUN) that regulate age-related DEGs. The NF-κB (RELA/NFKB1) signaling is a central driver of inflammatory gene expression and the maintenance of the senescent state.[21,22] STAT3 and STAT6 serve as primary mediators of cytokine signaling and contribute to chronic, low-grade inflammation (“inflammaging”) that increases with age.[23] The IRF1 is essential for antiviral defense and immune regulation, while JUN plays a role in proliferation and cellular stress responses. Our results highlight that these TFs may have a crucial role in the transcriptional program underlying immune aging.

Our enrichment analysis showed that several components of the PI3K/AKT signaling pathway were among the DEGs, emphasizing the pathway's importance in the aging process. This pathway is a well-known regulator of metabolism, cell growth, and survival, and it plays a central role in insulin signaling and T-cell development. However, as the body ages, this pathway becomes dysregulated, leading to insulin resistance, reduced metabolic flexibility, and an imbalance in immune cells. Notably, altering the activity of this pathway has been associated with increased lifespan in various model organisms. Therefore, the presence of PI3K/AKT-related genes in our analysis suggests that this pathway may be crucial in the metabolic and immune system changes that occur with aging.[2,18]

We also observed that TFs like TEAD4, FOXC1, and TLX3 target multiple miRNAs. TEAD4, which operates within the Hippo-YAP/TAZ-TEAD signaling pathway, has been associated with cellular senescence and tissue homeostasis, and its regulation of miRNAs may impact these processes.[24] These findings indicate that aging influences not only gene expression patterns but also complex TF-miRNA regulatory networks.

Immune cell infiltration analysis showed notable differences in iTreg, Tcm, and Tem between middle-aged and older adults. Prior research indicates that aging modifies the T-cell compartment in composition and function.[25] An increase in iTreg frequency may have dual and potentially conflicting effects: it could help reduce age-related chronic inflammation and tissue damage, but excessive regulatory activity might suppress protective immunity, impairing the ability to clear pathogens and diminishing vaccine responses in older adults.[26,27] During aging, Treg expansion reduces immune surveillance and increases susceptibility to infections and cancer.[28]

Alterations in Tcm and Tem subsets reflect adjustments in adaptive immune memory. Tcm cells, mostly residing in lymphoid tissues, enable quick expansion when re-encountering an antigen, while Tem cells, circulating in peripheral tissues, deliver immediate effector responses. As people age, the balance often

shifts toward a Tem-dominant profile, which is linked to a decreased ability to proliferate and generate new memory responses.[29] Our findings show significant shifts in these subsets, indicating an altered memory T-cell landscape that may impair recall responses and reduce adaptability to new antigens in older adults.

These changes in immune cell distribution indicate a complex reorganization of immune function with age, characterized by increased regulatory activity and qualitative and quantitative changes in memory T-cell populations. This interaction may account for the paradoxical coexistence of chronic low-grade inflammation and declining protective immunity in older adults.

Collectively, the PBMC-derived molecular signatures identified in this study, including NF-κB/STAT-associated DEGs and candidate miRNAs such as miR-223-3p, miR-19a/b, and miR-26b, offer promising minimally invasive biomarkers for guiding anti-aging strategies and monitoring pharmacodynamic responses. Emerging evidence highlights the therapeutic importance of miRNAs in aging regulation; for example, miR-34a has been linked to controlling nicotinamide adenine dinucleotide metabolism and endothelial senescence, with targeting miR-34a restoring sirtuin 1 and improving metabolic dysfunction in aging models.[30] Consistent with cross-species comparative evidence, conserved longevity pathways (GH/IGF-1/FOXO [Growth Hormone/Insulin Like Growth Factor 1/Forkhead Box O], TOR/S6K [Target of rapamycin/Ribosomal S6 kinase], sirtuins, AMPK [Adenosine monophosphate-activated protein kinase]) and interventions such as rapamycin, metformin, and dietary or methionine restriction have been shown to influence and moderately extend lifespan in model organisms.[31] Similarly, caloric restriction and rapamycin therapy have been demonstrated to slow epigenetic aging clocks and reverse DNA methylation patterns associated with biological aging, reinforcing the translational potential of molecular markers in aging treatments.[32] Additionally, our discovery of the involvement of the Hippo-YAP/TAZ-TEAD signaling axis aligns with its established role in cellular senescence and tissue maintenance, with recent work showing that pharmacologically disrupting YAP/TAZ-TEAD can influence proliferative and senescence-related processes in preclinical models.[33] However, applying these findings clinically remains challenging due to inflammaging being a system-wide phenomenon, and current therapies, including senolytics or Hippo pathway modulation, have shown inconsistent results across various biomarkers.[34]

Methodologically, our study has some limitations, as it presents *in silico* evidence for the suggested biomarkers related to PBMC-specific transcriptomic data, and it lacks experimental validation for *in silico*-predicted regulators. These limitations highlight the need for future large-scale, multi-omics, and longitudinal studies that incorporate functional assays to validate the causal significance and therapeutic potential of these proposed biomarkers.

In conclusion, key TFs such as NF-κB and members of the STAT family, along with aging-associated miRNAs that target critical immune genes, create an interconnected regulatory network. Understanding these networks could help develop targeted strategies to prevent or reverse age-related immune decline.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Conceptualization, methodology, formal analysis, investigation, data curation, visualization, writing-original draft: F.A.B.; Conceptualization, supervision, project administration, writing-review & editing: D.P.

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REFERENCES

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. Cell 2023;186:243-78. doi: 10.1016/j.cell.2022.11.001.
2. Li Y, Tian X, Luo J, Bao T, Wang S, Wu X. Molecular mechanisms of aging and anti-aging strategies. Cell Commun Signal 2024;22:285. doi: 10.1186/s12964-024-01663-1.

3. Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA, et al. Immunosenescence and inflamm-aging as two sides of the same coin: Friends or foes? Front Immunol 2018;8:1960. doi: 10.3389/fimmu.2017.01960.
4. Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S. Inflammaging and 'Garb-aging'. Trends Endocrinol Metab 2017;28:199-212. doi: 10.1016/j.tem.2016.09.005.
5. Wang Z, Saxena A, Yan W, Uriarte SM, Siqueira R, Li X. The impact of aging on neutrophil functions and the contribution to periodontitis. Int J Oral Sci 2025;17:10. doi: 10.1038/s41368-024-00332-w.
6. Noh JY, Han HW, Kim DM, Giles ED, Farnell YZ, Wright GA, et al. Innate immunity in peripheral tissues is differentially impaired under normal and endotoxic conditions in aging. Front Immunol 2024;15:1357444. doi: 10.3389/fimmu.2024.1357444.
7. Butcher SK, Chahal H, Nayak L, Sinclair A, Henriquez NV, Sapey E, et al. Senescence in innate immune responses: reduced neutrophil phagocytic capacity and CD16 expression in elderly humans. J Leukoc Biol 2001;70:881-6.
8. Sommer C, Reamon-Buettner SM, Niehof M, Hildebrand CB, Braun A, Sewald K, et al. Age-dependent inflammatory response is altered in an ex vivo model of bacterial pneumonia. Respir Res 2024;25:15. doi: 10.1186/s12931-023-02609-w.
9. Peters MJ, Joehanes R, Pilling LC, Schurmann C, Conneely KN, Powell J, et al. The transcriptional landscape of age in human peripheral blood. Nat Commun 2015;6:8570. doi: 10.1038/ncomms9570.
10. Noren Hooten N, Fitzpatrick M, Wood WH 3rd, De S, Ejiogu N, Zhang Y, et al. Age-related changes in microRNA levels in serum. Aging (Albany NY) 2013;5:725-40. doi: 10.18632/aging.100603.
11. Connolly E, Pan T, Aluru M, Chockalingam S, Dhere V, Gibson G. Loss of immune cell identity with age inferred from large atlases of single cell transcriptomes. Aging Cell 2024;23:e14306. doi: 10.1111/acel.14306.
12. Li W, Zhang Z, Kumar S, Botey-Bataller J, Zoodsma M, Ehsani A, et al. Single-cell immune aging clocks reveal inter-individual heterogeneity during infection and vaccination. Nat Aging 2025;5:607-21. doi: 10.1038/s43587-025-00819-z.
13. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, et al. g:Profiler: A web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res 2019;47:W191-8. doi: 10.1093/nar/gkz369.
14. Miao YR, Zhang Q, Lei Q, Luo M, Xie GY, Wang H, et al. ImmuCellAI: A unique method for comprehensive t-cell subsets abundance prediction and its application in cancer immunotherapy. Adv Sci (Weinh) 2020;7:1902880. doi: 10.1002/advs.201902880.
15. Tokar T, Pastrello C, Rossos AEM, Abovsky M, Hauschild AC, Tsay M, et al. mirDIP 4.1-integrative database of human microRNA target predictions. Nucleic Acids Res 2018;46:D360-70. doi: 10.1093/nar/gkx1144.
16. Han H, Cho JW, Lee S, Yun A, Kim H, Bae D, et al. TRRUST v2: An expanded reference database of human and mouse transcriptional regulatory interactions. Nucleic Acids Res 2018;46:D380-6. doi: 10.1093/nar/gkx1013.
17. Chen Y, et al. Role of miR-223 in immune regulation and aging-related diseases. Front Immunol 2022;13:853421. doi: 10.3389/fimmu.2022.853421.
18. Grillari J, Hackl M, Grillari-Voglauer R. miR-17-92 cluster: ups and downs in cancer and aging. Biogerontology 2010;11:501-6. doi: 10.1007/s10522-010-9272-9.
19. Zhou J, Li Z, Huang Y, Ju W, Wang D, Zhu X, et al. MicroRNA-26a targets the mdm2/p53 loop directly in response to liver regeneration. Int J Mol Med 2019;44:1505-1514. doi: 10.3892/ijmm.2019.4282.
20. Yan J, Chen S, Yi Z, Zhao R, Zhu J, Ding S, et al. The role of p21 in cellular senescence and aging-related diseases. Mol Cells 2024;47:100113. doi: 10.1016/j.mocell.2024.100113.
21. Haga M, Okada M. Systems approaches to investigate the role of NF-κB signaling in aging. Biochem J 2022;479:161-83. doi: 10.1042/BCJ20210547.
22. Jurk D, Wilson C, Passos JF, Oakley F, Correia-Melo C, Greaves L, et al. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nat Commun 2014;2:4172. doi: 10.1038/ncomms5172.
23. Samson LD, Engelfriet P, Verschuren WMM, Picavet HSJ, Ferreira JA, de Zeeuw-Brouwer ML, et al. Impaired JAK-STAT pathway signaling in leukocytes of the frail elderly. Immun Ageing 2022;19:5. doi: 10.1186/s12979-021-00261-w.
24. Elster D, von Eyss B. Hippo signaling in regeneration and aging. Mech Ageing Dev 2020;189:111280. doi: 10.1016/j.mad.2020.111280.
25. Nikolich-Žugich J. The twilight of immunity: Emerging concepts in aging of the immune system. Nat Immunol 2018;19:10-9. doi: 10.1038/s41590-017-0006-x.
26. Salam N, Rane S, Das R, Faulkner M, Gund R, Kandpal U, et al. T cell ageing: effects of age on development, survival & function. Indian J Med Res 2013;138:595-608.
27. Pinti M, Appay V, Campisi J, Frasca D, Fülöp T, Sauce D, et al. Aging of the immune system: Focus on inflammation and vaccination. Eur J Immunol 2016;46:2286-301. doi: 10.1002/eji.201546178.
28. Jagger A, Shimojima Y, Goronzy JJ, Weyand CM. Regulatory T cells and the immune aging process: a mini-review. Gerontology 2014;60:130-7. doi: 10.1159/000355303.

29. Goronzy JJ, Weyand CM. Mechanisms underlying T cell ageing. Nat Rev Immunol 2019;19:573-83. doi: 10.1038/s41577-019-0180-1.
30. Turko R, Hajja A, Magableh AM, Omer MH, Shafqat A, Khan MI, et al. The emerging role of miRNAs in biological aging and age-related diseases. Noncoding RNA Res 2025;13:131-52. doi: 10.1016/j.ncrna.2025.05.002.
31. Ma S, Gladyshev VN. Molecular signatures of longevity: Insights from cross-species comparative studies. Semin Cell Dev Biol 2017;70:190-203. doi: 10.1016/j.semcdb.2017.08.007.
32. Zhai J, Kongsberg WH, Pan Y, Hao C, Wang X, Sun J. Caloric restriction induced epigenetic effects on aging. Front Cell Dev Biol 2023;10:1079920. doi: 10.3389/fcell.2022.1079920.
33. Elster D, von Eyss B. Hippo signaling in regeneration and aging. Mech Ageing Dev 2020;189:111280. doi: 10.1016/j.mad.2020.111280.
34. Abdellatif M, Schmid ST, Fuerlinger A, Kroemer G. Anti-ageing interventions for the treatment of cardiovascular disease. Cardiovasc Res 2025;121:1524-36. doi: 10.1093/cvr/cvae177.