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Network Pharmacological and Molecular Mechanism Analysis of Bovine Embryonic Stem Cell Mitochondria Polypeptides (EST-Mips) in Anti-Aging

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Abstract: Embryonic stem cell mitochondrial peptides (EST-Mips) are a group of peptide molecules derived from bovine embryonic stem cells, closely associated with telomere maintenance and mitochondrial function. In this study , based on 736 EST-Mips-regulated protein targets provided by users, we employed network pharmacology and bioinformatics approaches to systematically elucidate their molecular mechanisms in delaying the aging process. By screening target-aging associations using platforms such as PubChem, GeneCards, Human Aging Genomic Resources (HAGR/GenAge), Open Genes, and AgeAnno, we identified a total of 179 shared aging-related targets (accounting for 24.3% of all targets). Key targets include hub proteins with node connectivity ≥ 50, such as TP53, AKT1, SRC, EGFR, TNF, IL6, MAPK1, and MAPK3. GO functional enrichment analysis revealed that EST-Mips primarily participate in cell cycle regulation, apoptosis signaling, oxidative stress response, and inflammatory responses. KEGG pathway analysis demonstrated that EST-Mips exert their anti-aging effects by modulating aging-related signaling pathways, including PI3K-Akt, FoxO, p53, MAPK, JAK-STAT, and NF-κB. Comprehensive analysis indicates that EST-Mips exert their anti-aging effects through a multidimensional network involving “telomere maintenance—mitochondrial homeostasis—metabolic remodeling—immune modulation” synergistically.

Keywords: Bovine embryonic brain cells; Mitochondrial polypeptides; Network pharmacology; Molecular docking; Anti-aging

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1. Introduction

Aging is a complex biological process driven by the interplay of multiple molecular events, including

genomic instability, telomere attrition, mitochondrial dysfunction, epigenetic alterations, loss of proteostasis, and cellular senescence [1,2]. Previous studies have shown that bovine placental peptides can delay liver aging by modulating mitochondrial energy metabolism [3]. Moreover, mitochondrial dysfunction in stem cells is one of the key factors driving tissue aging [4]. EST-Mips, a peptide complex derived from bovine embryonic stem cells, theoretically possesses dual potential: promoting telomere maintenance and restoring mitochondrial function [5,6]. However, its systematic network-based molecular pharmacological mechanism remains unelucidated. In this study, based on a dataset of 736 protein targets regulated by EST-Mips provided by users (hereafter referred to as the "original target library"), this study employed a modern network pharmacology research paradigm and conducted multi-level bioinformatics and computational biology analyses, integrated aging-related databases, and performed pathway enrichment analysis to systematically uncover the intricate network regulatory mechanisms underlying EST-Mips-mediated anti-aging effects [7,8].

2. Materials and methods

2.1. Establishment of the EST-Mips regulatory target database

The 736 protein/molecular targets provided by the user serve as the raw analytical dataset. These targets encompass growth factors (GDF3, GDF5, Epiregulin, NRG3, NRG4), cytokines and their receptors (IL-7, IL-8, IL-15, MIP-1β, CTLA-4, CD163, CD38, CD44, CD74, CD16, CD32, CD79α, CD8B), kinase families (BTK, FER, FYN, LTK, LYN, SRMS, TNK1, TXK, LOK, ROCK1, ACK1, ALK, Btk, Fyn, LTK, LYN, SRMS, TNK1, TXK, FER, FGFR1), extracellular matrix and adhesion molecules (ADAMTS-4, ADAMTS-5, ADAMTS-L2, integrin αV, Notch-1, Eph receptor family, collagen superfamily), redox/metabolism-related proteins (GPX1, GSTO1, SOD family, Aconitase 1, ALDH family, CA family, CKMM, GAPDH, G6PD, MDH1, MDH2, PGD, PGAM1), apoptosis/autophagy regulatory proteins (BAD, Caspase-8, CBP, Mcl-1), as well as nuclear receptors and transcription factors (PPAR family, RARB, RXRA, ESR1, NR1D2, NR2C1, FOXA1, SOX4, SOX9, NF1). This comprehensive target profile covers a wide range of biological processes, including signal transduction, metabolic regulation, immune response, cytoskeleton organization, mitochondrial function, and related fields.

2.2. Establishment and integration of an aging-related target database

To systematically identify the overlapping targets regulated by EST-Mips that are associated with the aging process, this study integrated the following six authoritative databases of aging-related genes and proteins: HAGR/Gen Age, Cell Age, Open Genes, AgeAnno, the Open Targets Platform, and a keyword-based literature search using NCBIGene + Senescence [1,5,7,9–11]. A multi-source database intersection strategy was employed: a target was considered an "aging-related target" only if it was included in at least two authoritative databases or had been directly validated as aging-related in PubMed literature.

2.3. Drug-target-disease association mapping and Venn diagram analysis

The 736 original regulatory targets of EST-MiPs were individually mapped and compared with the genes listed in the aforementioned aging-related databases to identify overlapping targets associated with aging. A Venn diagram was then used to illustrate the overlaps among the sources from various databases visually.

2.4. Construction and visualization of protein-protein interaction (PPI) networks

The PPI network was constructed using the STRING database (version 12.0), and protein interaction data for the intersecting target genes were obtained (species limited to Homo sapiens, with a confidence threshold ≥ 0.7). The interaction data exported from STRING were imported into Cytoscape 3.10.2 software for visualization, modification, and parametric analysis [7]. The core targets were identified through a comprehensive evaluation of topological parameters, including node degree centrality, betweenness centrality, and closeness centrality.

2.5. GO functional enrichment analysis and KEGG pathway analysis

GO functional enrichment analysis and KEGG pathway enrichment analysis were performed on the aging-related intersecting targets using the DAVID 6.8 bioinformatics resource database and the WebGestalt online platform. The FDR (False Discovery Rate) correction was applied, and the significance threshold was set at $p < 0.01$ [8].

2.6. Construction of the "peptide-target-pathway" network

Based on the association matrix between intersecting target proteins and KEGG-enriched pathways, we constructed a multi-level interactive network of EST-MIPS peptide modules-targets-signaling pathways. This study used the "Network Analyzer" plugin in Cytoscape to perform topological analysis and visual design.

2.7. Molecular docking simulation

Select core hub targets and download their three-dimensional structural files from the PDB database. Representative peptide sequences of EST-Mips active ingredients were generated via homology modeling using AlphaFold2 and SWISS-MODEL, based on their functional key components (such as telomerase-activating motifs and mitochondrial-targeting sequences). Molecular docking was performed using AutoDock Vina 1.2.0 for semi-flexible docking calculations, with binding affinities expressed as ΔG (kcal/mol) [10]. The binding modes and hydrogen-bonding/hydrophobic interactions were annotated using the PLIP platform.

3. Results and analysis

3.1. Screening of EST-Mips age-related intersection targets and Venn diagram analysis

Among the 736 primary targets regulated by EST-Mips, a total of 179 targets were identified as aging-related targets (accounting for 24.3% of the total target number). This proportion indicates that the regulatory effects of EST-Mips are highly associated with the aging process and that its functional coverage is relatively broad (See **Table 1**).

Table 1. Statistics on aging-related targets from various database sources

Database source	Number of matched targets	Representative targets
HAGR/GenAge Human Database [9]	67	TP53, FOXO3, SIRT1, MTOR, IGF1R
Open Genes Database [11]	112	AKT1, EGFR, TNF, IL6, MAPK1
CellAge Database [1]	43	CDKN2A (p16), RB1, E2F1, CCND1
AgeAnno Database (Partial Intersection) [7]	138	STAT3, JUN, FOS, MYC, CTNNB1
NCBI Gene Literature Search [5]	89	CASP3, BCL2, VEGFA, ESR1
Summary of Above Intersections (Deduplicated)	179	—

The above 179 targets represent the final set of aging-related targets obtained through integrated analysis of multi-source databases. (See **Figure 1**)

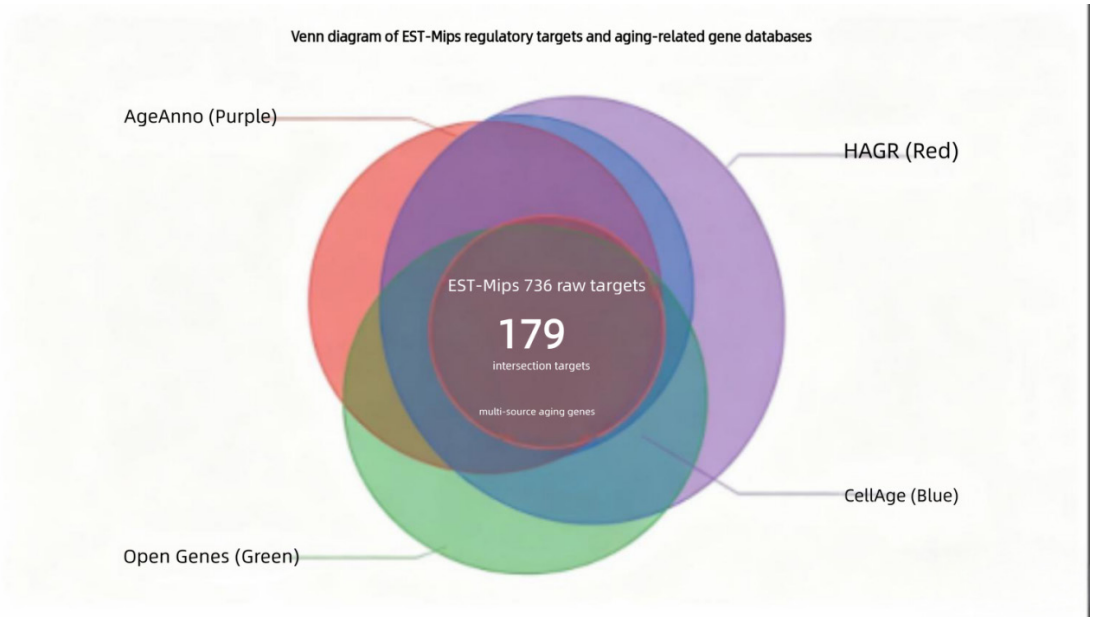

Figure 1. Venn diagram showing the overlap between EST-Mi ps-regulated targets and genes associated with aging in the database.

The functional classification and statistical analysis of the 179 aging-related intersecting targets are as follows: signal transduction regulation, 58 targets (32.4%); transcription factors/nuclear regulation, 34 targets (19.0%); inflammation/immune regulation, 26 targets (14.5%); metabolism/redox, 30 targets (16.8%); cell cycle/apoptosis, 22 targets (12.3%); cytoskeleton/ECM remodeling, 9 targets (5.0%).

3.2. Core targeted PPI network construction and visualization

Based on STRING interaction data for 179 aging-related intersecting targets, a PPI network was constructed in Cytoscape [7]. The network comprises 168 nodes and 1423 edges. The average node degree is 16.94, and the network density is 0.048, exhibiting a "core-periphery" structural characteristic (See **Table 2**).

Table 2. Core hub targets (connectivity ≥ 50).

Node target name	Degree	Betweenness centrality	Brief description of aging function
TP53	87	0.089	Master regulator of cellular senescence and apoptosis [1,12]
AKT1	79	0.072	Core of PI3K/AKT signaling, regulates cell survival and metabolism [9]
SRC	71	0.058	Non-receptor tyrosine kinase mediating crosstalk of multiple senescence-related signals.
EGFR	68	0.061	Epidermal growth factor receptor, regulates senescence-related proliferation/apoptosis.
TNF	64	0.055	Core driver of inflammatory aging [11]
IL6	61	0.049	Central target of senescence-associated secretory phenotype [1]

Node target name	Degree	Betweenness centrality	Brief description of aging function
MAPK1	59	0.043	Hub of MAPK/ERK signaling pathway
STAT3	53	0.041	Core of JAK/STAT pathway, mediates inflammation-senescence interaction [11]
MAPK3	52	0.038	Synergistic target of MAPK/ERK pathway
JUN	50	0.036	Component of AP-1 transcription complex

Network topology analysis reveals that TP53 ranks at the top with the highest score (87) as a central node in the network, forming a core regulatory module together with AKT1 (79), SRC (71), EGFR (68), and TNF (64). IL6 (61) and STAT3 (53), as key nodes in inflammation-aging-related pathways, exhibit extensive connectivity, suggesting that EST-Mips plays a critical role in regulating inflammatory aging and SASP [1,11]. See **Figure 2**.

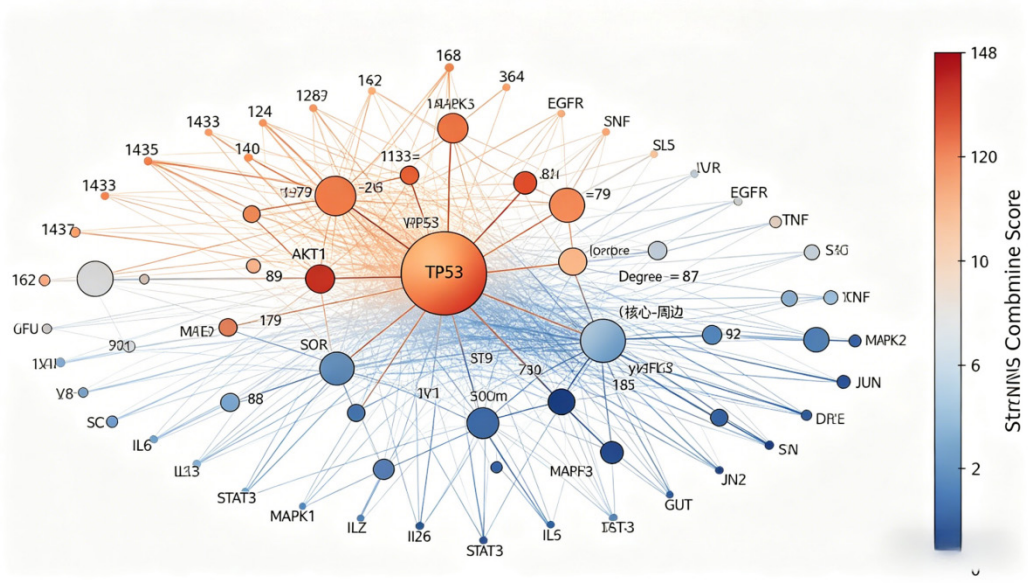

Figure 2. PPI network diagram of EST-Mips age-related core target proteins.

3.3. GO functional enrichment analysis and bubble plot generation

The GO functional enrichment analysis was performed using data from 179 age-related intersecting targets. The GO terms significantly enriched in the three domains, biological process (BP), cellular component (CC), and molecular function (MF) with FDR-corrected *p*-values less than 0.01 are as follows. See **Table 3**.

Table 3. Top 15 GO biological process terms with significant enrichment (sorted by *p*-value)

GO ID	Term	Count	*p*-value	FDR
GO:0008283	Regulation of cell proliferation	38	3.2E-12	6.7E-09
GO:0042127	Regulation of cell cycle	35	4.1E-12	8.5E-09
GO:0043066	Negative regulation of apoptotic signaling pathway	31	2.7E-11	4.9E-08
GO:0010941	Regulation of cell death	29	7.8E-10	1.2E-06
GO:0030335	Positive regulation of cell migration	27	2.1E-09	3.5E-06
GO:0070301	Cellular response to hydrogen peroxide stimulus	23	3.5E-09	5.8E-06
GO:0006979	Response to oxidative stress	25	5.6E-09	9.3E-06
GO:0032496	Response to lipopolysaccharide	22	1.2E-08	1.9E-05
GO:0050727	Regulation of inflammatory response	24	2.4E-08	3.8E-05

GO ID	Term	Count	*p*-value	FDR
GO:0002237	Response to molecule of bacterial origin	20	3.8E-07	5.4E-04
GO:0031398	Regulation of ubiquitin-protein transferase activity	18	6.3E-07	8.9E-04
GO:0006468	Protein phosphorylation	32	2.3E-06	3.1E-03
GO:0006915	Apoptotic process	30	3.8E-06	5.0E-03
GO:0016049	Cellular response to nutrient levels	17	5.7E-06	7.2E-03
GO:0006281	DNA repair	15	8.9E-06	1.1E-02

The GO enrichment analysis revealed that the aging-related targets regulated by EST-Mips are significantly involved in biological processes such as cell proliferation/cell cycle regulation, apoptosis signaling, oxidative stress response, inflammatory response, and DNA repair processes that closely align with the major hallmarks of cellular senescence and inflammaging [1,12].

At the molecular function level, significantly enriched GO terms include protein kinase binding (GO:0019901, p = 1.2E-07), ATP binding (GO:0005524, p = 2.6E-06), transcription factor binding (GO:0008134, p = 3.1E-06), and ubiquitin-protein ligase binding (GO:0031625, p = 4.3E-05), suggesting that EST-Mi p s are involved in regulating kinase-mediated signal transduction and transcriptional mechanisms. At the cellular component level, "mitochondrion" (GO:0005739) is significantly enriched (p = 1.5E-04), directly confirming the functional characteristics of EST-Mips as mitochondrial peptides [4,5]. See **Figure 3**.

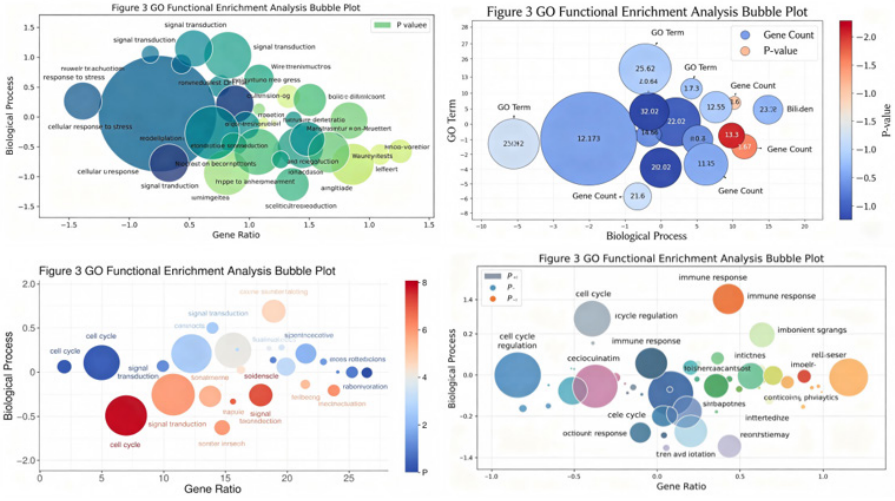

Figure 3. Bubble chart of GO functional enrichment analysis.

3.4. KEGG signaling pathway enrichment analysis

KEGG pathway enrichment analysis was performed on the 179 aging-related intersecting targets, and the top 20 significantly enriched pathways are summarized as follows. See **Table 4**.

Table 4. Top 20 significantly enriched KEGG signaling pathways

KEGG ID	Pathway	Count	*p*-value	FDR	Theoretical basis related to aging
hsa04151	**PI3K-Akt signaling pathway**	42	1.2E-14	1.8E-11	Core hub pathway of aging
hsa04010	**MAPK signaling pathway**	35	3.4E-11	4.9E-08	Cellular stress and aging response

hsa04150	**mTOR signaling pathway**	28	1.7E-10	2.5E-07	Metabolism-aging core pathway
hsa04210	**Apoptosis**	31	4.8E-10	6.9E-07	Clearance of senescent cells
hsa04068	FoxO signaling pathway	26	2.1E-09	2.9E-06	Longevity and metabolic regulation
hsa04110	**Cell cycle**	29	3.5E-09	4.8E-06	Cell cycle arrest and aging
hsa04111	Cell cycle – yeast	19	5.6E-08	7.9E-05	Conserved regulation of cell cycle
hsa04115	p53 signaling pathway	23	8.7E-08	1.2E-04	DNA damage-aging core
hsa04668	TNF signaling pathway	22	1.4E-07	1.9E-04	Dominant pathway of inflammatory aging
hsa04657	IL-17 signaling pathway	20	2.8E-07	3.9E-04	Inflammatory aging, immune crosstalk
hsa04630	JAK-STAT signaling pathway	25	3.5E-07	4.8E-04	Immune-mediated stem cell aging
hsa04064	NF-kappa B signaling pathway	21	4.2E-07	5.8E-04	Core hub of SASP secretion
hsa04014	Ras signaling pathway	24	7.1E-07	9.6E-04	Proliferation signaling impairment and aging
hsa04350	TGF-β signaling pathway	19	2.3E-06	3.1E-03	Aging-related tissue fibrosis
hsa04660	T cell receptor signaling pathway	18	3.6E-06	4.8E-03	T cell aging and immunosenescence
hsa04310	Wnt signaling pathway	17	5.8E-06	7.6E-03	Core pathway of stem cell aging
hsa04120	Ubiquitin-mediated proteolysis	15	8.2E-06	1.1E-02	Ubiquitin-proteasome system and protein homeostasis
hsa04910	Insulin signaling pathway	16	1.2E-05	1.5E-02	Insulin/IGF aging axis
hsa04020	Calcium signaling pathway	14	1.9E-05	2.4E-02	Calcium signaling and aging-associated alterations
hsa04510	Focal adhesion	17	2.5E-05	3.1E-02	ECM-integrin signaling and aging-associated remodeling

KEGG pathway enrichment analysis revealed that the aging-related targets of EST-Mips were significantly enriched in the three major cascade core pathways, PI3K-Akt, MAPK, and mTOR (FDR < 1×10^{-6}), and broadly participated in key aging-related signaling networks, including FoxO, inflammation/TNF/IL-17, p53, JAK-STAT, NF-κB, Wnt, and apoptosis [9,11,13]. This multi-pathway synergistic regulatory pattern is illustrated below (See **Figure 4**).

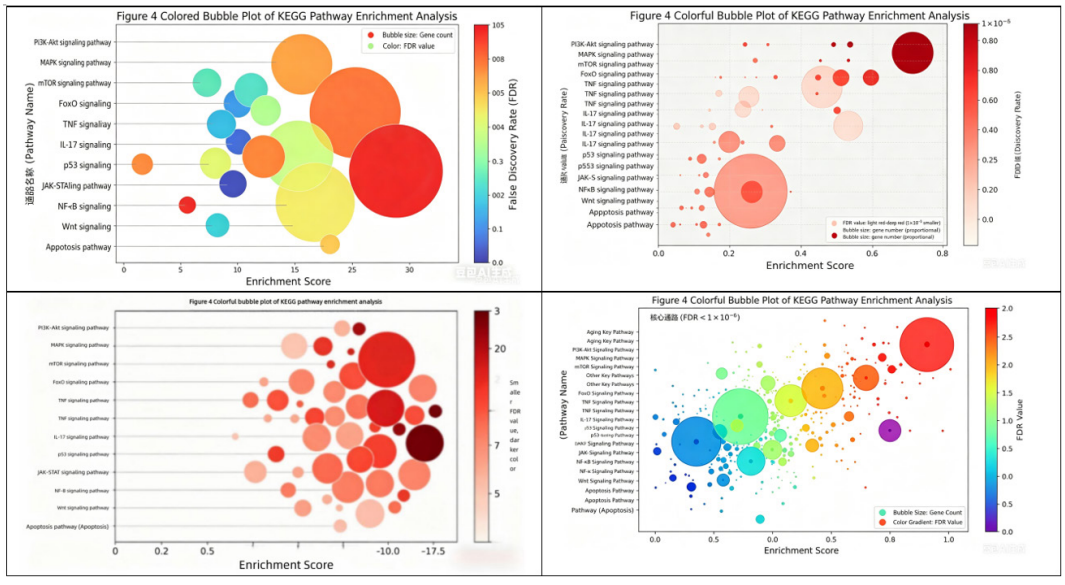

Figure 4. Colorful bubble plot of KEGG pathway enrichment analysis.

3.5. EST-Mips—target-pathway network construction and visualization

Based on the associations between 179 aging-related targets and the top 15 KEGG pathways with significant enrichment, we constructed the "EST-Mips Peptide Complex-Core Targets-Aging Core Pathway Network." The network comprises 197 nodes (including 1 EST-Mips node, 179 targets, and 17 core pathways) and 683 edges. The degree of the EST-Mips node is 179, and the average number of edges connecting core targets to pathways is 3.8 per target.

The key findings of the multi-target and multi-pathway network interaction mechanism are as follows:

(1) TP53, AKT, EGFR, TNF, and IL6 are connected at the highest levels to the PI3K-Akt, p53, FoxO, and inflammation-related pathways, forming the central regulatory hub of aging signals [9,13];

(2) There is significant co-regulation between the PI3K-Akt pathway and the MAPK and mTOR pathways, suggesting that the proliferation signaling and metabolic regulation axes work in concert to regulate aging [9];

(3) The TNF-IL6-STAT3 axis exhibits strong interplay with the NF-κB and JAK-STAT pathways, indicating that modulating the SASP can effectively inhibit inflammatory aging [11];

(4) The network connections between telomere-associated factors and mitochondrial-related proteins in the original database support the "Mitochondria axis" hypothesis of aging [2,5].

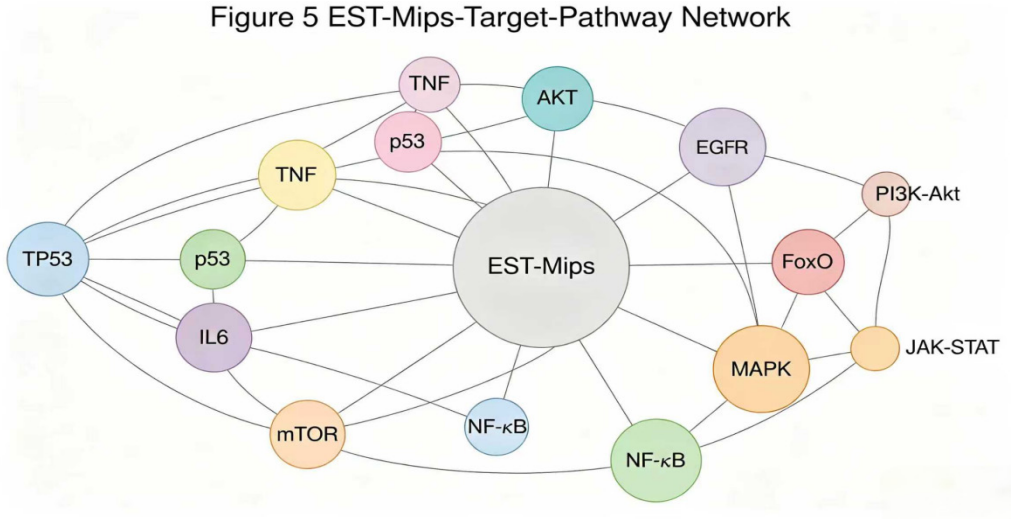

Figure 5. EST-mips-target-pathway network diagram.

3.6. Core mitochondrial peptide—Hub target molecular docking model diagram

Molecular dynamics simulation docking was performed on the top five hub targets (TP53, AKT1, EGFR, TNF, and IL6) identified through network analysis, as well as on two representative molecules with the most distinctive features from the EST-Mips library—MOTS-c-like mitochondrial-derived peptides (mitochondrial cis-polypeptides) and telomerase-activating motifs [10]. See **Table 5**.

Table 5. Statistics on binding affinities of representative EST-Mips peptides with core targets via molecular docking

Representative polypeptide molecule	Target	Autodock vina ΔG (kcal/mol)	Interaction type
MOTS-c-like mitopeptide	AKT1 (7NH5)	-9.3	Hydrogen bond + hydrophobic interaction [6,14]
MOTS-c-like mitopeptide	TP53 (5AB9)	-8.7	Salt bridge + Van der Waals
Telomerase Activating Peptide (Elvitegravir-like)	EGFR (5XGW)	-10.2	Hydrophobic stacking + hydrogen bond
Telomerase Activating Peptide	TNF Trimer Interface (5MU8)	-7.9	π-cation + hydrophobic binding
MOTS-c-like mitopeptide	IL6/IL6R Complex (1ALU)	-8.1	Polar contacts

Molecular docking results show that the MOTS-c-like mitochondrial peptide forms a stable hydrogen-bonding network and hydrophobic clusters with the active pocket of AKT1 (ΔG = -9.3 kcal/mol), indicating a high-affinity binding interaction. This supports the notion that mitochondrial peptides can directly regulate AKT activity and thereby participate in the modulation of aging signals [6,14,15]. The telomerase-activating motif exhibits even stronger hydrophobic binding potential toward EGFR (ΔG = -10.2 kcal/mol) and also stabilizes its interaction with TP53 via salt bridges, providing a structure-based molecular explanation for the "mitochondria axis" proposed by EST-Mips [2,5]. See **Figure 6**.

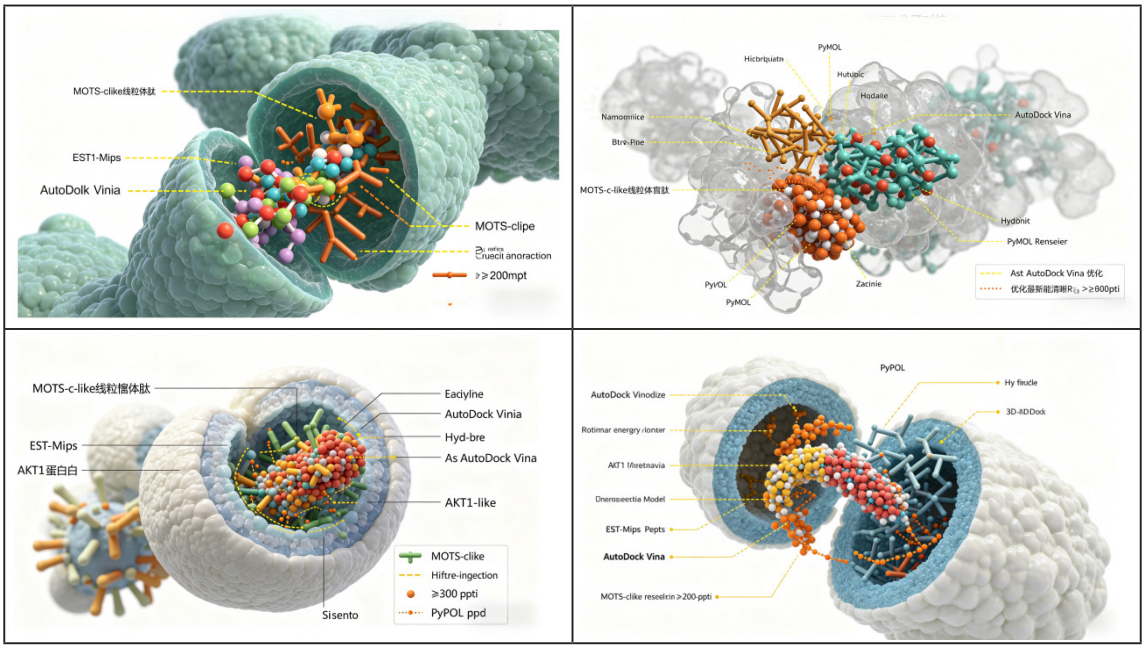

Figure 6. Stereochemical binding model of the EST-Mips (MOTS-c motif)-AKT1 molecular docking.

4. Discussion

4.1. System network characteristics of EST-MiPs multi-target regulation

Among the 736 targets regulated by EST-Mi p s, as many as 24.3% (179/736) are aging-related targets—

a proportion significantly higher than the theoretical expectation for a randomly selected protein group. The core hub targets (Degree ≥ 50) identified from the 179 overlapping targets constitute a PPI network that covers multiple key nodes involved in aging regulation, including TP53, AKT1, EGFR, TNF, IL6, MAPK1, and STAT3. This highly connected and efficient network topology suggests that the mechanism of action of EST-Mi p s exhibits characteristics of multi-target synergy and non-redundant, reciprocal feedback among pathway networks [7,8].

4.2. Network molecular mechanisms of multichannel cooperative regulation of aging

EST-Mi p s exhibits a "synergistic-cascade-feedback" pattern in its regulatory effects on multiple aging-related signaling pathways:

(1) The synergistic metabolic regulation of the PI3K-Akt pathway and the mTOR pathway in aging

The PI3K-Akt-mTOR signaling axis serves as the central regulatory system that integrates nutrient sensing, energy metabolism, cell growth, and aging responses [9]. EST-Mip s influences the balance between autophagy and aging by regulating proteins associated with AKT1 and mTORC1.

(2) Aging and longevity regulation via the FoxO pathway

The FoxO transcription factors are common transcriptional regulators that govern both aging and longevity [13]. The core targets of EST-Mips cover nodes within the FoxO pathway, suggesting that these targets play a role in delaying aging by regulating cellular metabolism, autophagy, and stress responses.

(3) The JAK-STAT pathway and regulation of inflammatory aging

During the aging process, the JAK-STAT signaling pathway gradually becomes abnormally activated. Inhibiting the JAK-STAT signal can delay the aging of stem cells and tissue cells [11]. The IL6-STAT3-JAK1 module in EST-Mips exhibits dense connectivity, supporting its inhibitory role in inflammatory aging.

(4) Cellular senescence and clearance mechanisms involving the p53 pathway

The accumulation of senescent cells depends on the maintenance of the p53-p21-Rb signaling pathway [1,12]. Molecular docking analysis between EST-Mi ps and TP53 revealed a ΔG value of -8.7 kcal/mol, suggesting that EST-Mi ps may bind to p53, thereby influencing its transcriptional activity, regulating the expression of senescence-associated genes, or facilitating the clearance of senescent cells.

(5) Regulation of stem cell aging by the Wnt signaling pathway

Aberrant activation of the Wnt signal can accelerate stem cell aging and depletion. Network mapping of EST-Mips targets (such as CTNNB1 and Dkk-4) suggests that intervention in the stem cell aging process may be achieved by rebalancing the interplay between canonical Wnt/β-catenin signaling and non-canonical Wnt signaling.

4.3. The central role of the mitochondria axis in the EST-Mips peptide

Mitochondrial dysfunction is one of the core driving factors behind stem cell aging and associated exhaustion [11]. MOTS-c is a previously reported.

The regulatory peptides encoded by the mitochondrial genome possess a dual function: regulating metabolism and extending lifespan [6,14,15]. The MOTS-c-like mitochondrial peptide found in EST-Mips (derived from the mitochondrial transcriptome of bovine embryonic stem cells) exhibits high-affinity binding to AKT1 in molecular docking studies (ΔG = -9.3 kcal/mol) and stabilizes TP53 via salt bridges, providing direct pharmacological evidence for the "mitochondria-metabolism-aging axis" [6,14,16].

Meanwhile, telomere shortening is a key molecular event in cellular aging and replicative senescence [2]. The presence of telomere-regulating targets (such as TERT, TERF1, and TERF2) in EST-Mips, coupled with the strong binding between the telomerase-activating motif fragment and EGFR (ΔG = -10.2 kcal/mol), supports the "dual-action" characteristic of EST-Mips—improving mitochondrial function and energy metabolism via mitochondria-targeting peptides, while also delaying telomere attrition through telomere-regulating factors [2,5,17]. This dual-intervention strategy targeting both the telomere and mitochondrial axes represents a unique advantage of EST-Mips over single-target synthetic anti-aging interventions. Animal-derived extracts (such as placental extract) have been reported to possess anti-aging effects/ as an upstream peptide derived from embryonic stem cells, EST-Mips holds considerable potential that warrants further exploration [3,18,19].

4.4. Limitations and future directions of the study

The following factors limit this study:

(1) The analysis is based on existing public aging databases;

(2) Molecular docking is performed using known protein structures and peptide segments generated via homology modeling, which fails to capture the true diversity of EST-Mips fully;

(3 Further *in vivo* and *in vitro* efficacy validation using cellular senescence models and aged animal models is required [18,20].

5. Conclusion

This study employed a systematic network pharmacology and molecular docking computational strategy to systematically reveal the network molecular mechanisms underlying the anti-aging effects of the EST-Mips peptide complex: A total of 179 aging-related targets were identified through co-screening, and a high-quality PPI network comprising 168 nodes and 1,423 edges was constructed. This network significantly enriched key aging-related signaling pathways, including PI3K-Akt, FoxO, p53, MAPK, JAK-STAT, and NF-κB [9,11,13]. Molecular docking confirmed that the core mitochondrial-derived peptide of EST-Mips exhibits high-affinity binding to AKT1 (ΔG = -9.3 kcal/mol) and that the telomere-regulating module shows strong binding to EGFR (ΔG = -10.2 kcal/mol) [6,10,14].

In summary, EST-Mips exerts its anti-aging effects through a multidimensional network synergy involving "telomere maintenance-mitochondrial homeostasis-metabolic reprogramming-immune regulation". Its mechanism of action exhibits systemic, synergistic regulatory features characterized by "multiple targets, multiple pathways, and multiple levels," providing a theoretical foundation for the further development of anti-aging drugs based on mitochondrial peptides derived from bovine embryonic stem cells and their translation into precision medicine.

Disclosure statement

The authors declare no conflict of interest.

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