RESEARCH PAPER

Identification Of Genetic Biomarkers For Cellular Senescence In Mesenchymal Stem Cells

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ABSTRACT
This study aims to identify reliable biomarkers for senescence in mesenchymal stem cells (MSCs) derived from adipose tissue (AD), dental pulp (DP), and the umbilical cord (UMB). Data collection and rigorous preprocessing were followed by sequence alignment using the HISAT2 tool and gene counting with the featureCounts tool. Differential gene expression analysis, Gene set enrichment, network analysis, and pathway enrichment analyses were conducted. Gene interactions were analysed using the STRING database and Cytoscape software, incorporating the CytoHUBBA plugin for network ranking using various centrality measures. The Pathway analysis determined 20 up-regulated and 10 down-regulated genes in senescence pathways. In UMB samples, upregulated genes like PIK3CD and CCND3 (both are members of the AKT pathway), which promoted cellular stress and senescence. AD samples revealed the up-regulated expression from genes such as IGFBP3 and SERPINE1, related to IGF signalling and apoptosis. In DP samples NRAS and MAPK3 assist cell proliferation when they have a mutation it leads them to DNA damage. Downregulation of genes in UMB, being such examples as EIF4EBP1 that interferes with protein translation and cell cycle control thus leading to induced senescence. Downregulation of genes like CALML6 in AD (impairing stress responses) and DP exhibiting reduced expression involving immune function and oxidative stress response such as IL6. The molecular docking interactions of catechin, a known natural wound healing compound against the target proteins displayed good binding affinity with respective targets, especially with NRAS protein which hint towards its satisfactory role managing cellular senescence in MSCs. These findings reveal the important pathways of the senescence process and pinpoints the possible targets for the eradication of cellular age-related issues. This analysis of gene expression information provides valuable insights into the senescence process in different MSC types, paving the way for developing anti-senescence therapies pending further in-vitro validation..

Keywords: Mesenchymal stem cell, cellular senescence, differential gene expression, molecular docking, catechin.
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INTRODUCTION

Cellular senescence was essentially the issue of spontaneous cell cycle arrest that had been originally detected till was recognized as a response to stress in both in vitro and in vivo systems. Recent research also implicates senescence in physiological processes during development. The concept of cellular senescence began with Hayflick and Moorhead's 1961 study on human primary fibroblasts, aiming to establish reliable cell cultures for vaccine development and biotechnology. They observed that while tumor cell lines proliferated indefinitely, primary fibroblasts eventually ceased dividing, displaying a stable growth arrest despite stimulation attempts. This phenomenon linked cellular senescence to the aging process, suggesting a counting mechanism that triggers senescence, which has become a focal point in studying aging and cancer. Subsequent research revealed that cellular senescence acts as a barrier to tumorigenesis. Cells with activated oncogenes either undergo apoptosis or enter senescence, a stable cell cycle arrest. This discovery, supported by in vivo studies, positioned senescence as a critical anti-tumour mechanism. Further research identified senescence as a protective response to various stresses, preventing the proliferation of damaged cells.

Interestingly, senescence has also been observed during embryonic development, indicating its potential physiological role in tissue remodelling. This suggests that senescence may have originated as a developmental process, later adapted to respond to stress in adulthood. While the implications of senescence in health, aging, and disease continue to be intensely studied, its significance as a cellular response is indisputable (Da Silva-Álvarez and Collado 2016). Cellular senescence is tightly regulated by the p53 and pRB tumour suppressor pathways. Mutations in these pathways, or their regulators, are common in cancer cells. Components of these pathways, such as p16 and ARF,

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function as independent tumour suppressors, inhibiting oncogenes and cell proliferation promoters. Relationship between p53 and pRB pathways further shows their collaborative role in senescence. For example, pRB stops E2F1, whereas p53 raises p21 transcription, keeping pRB in its active state. Both pathways also govern cell fates other than senescence, including apoptosis and transient cell cycle arrest, depending on cell type and stimulus nature. The p53 pathway respond mainly to DNA damage, while the pRB pathway is stimulated by multiple stresses, often leads to the activation of p16. Despite some cells losing the ability to express p16, they remain sensitive to p53-mediated cellular senescence. Senescence mediated by the p16/pRB pathway is permanent, even if p53, p16, or pRB is inactivated subsequently, showing that it is a powerful secondary barrier against tumorigenesis (Campisi 2008). Mesenchymal stem cells (MSCs), known for their self-renewal and multi-lineage differentiation capabilities, the tissues such as the adipose tissue the umbilical cord, and dental pulp that these mesenchymal cells are derived from. These cells are promising for regenerative medicine because of their availability and their ability to function as therapeutics (Ding, Shyu and Lin, 2011). Adipose-derived MSCs from the adipose tissues, can be obtained for their regenerative properties as well as their potential applications in tissue repair (Vidal et al. 2012; Si et al. 2019). Dental pulp-derived MSCs (DPSCs) have similar traits and can be used in regenerative therapies (Chen et al. 2022). Umbilical cord-derived MSCs have immune-modulating properties, that enable them to work as allogeneic transplants (belonging to one donor and transferred to another donor) and immunotherapy (Nagamura-Inoue 2014; Stanko et al. 2014). Catechins are antioxidant polyphenols abundant in tea leaves, that have been getting attention as of their potential health benefits including; antimicrobial, antiviral, anti-inflammatory, anti-allergic, and anticancer properties. Their ability to enhance the absorption of beneficial compounds in food and skincare products mention their importance in dietary and topical applications. The stability of catechins and their safety level depict the potentials as super antioxidants in human health (Isemura 2019; Bae et al. 2020). Their wound-healing capacity is the reason it can directly interact with cellular senescence proteins.

The aim of the study is to identify genetic biomarkers responsible for cellular senescence in MSCs derived from adipose tissue, umbilical cord, and dental pulp. Understanding these biomarkers will enhance the therapeutic potential of MSCs in regenerative medicine and provide insights into the molecular mechanisms governing cellular senescence(Al-Azab et al. 2022; Kwiatkowska et al. 2023; Wang, Gao and Wang 2023).

METHODOLOGY

In this study, several bioinformatics tools and databases were used to analyse and interpret the genetic data related to cellular senescence in mesenchymal stem cells. GEO DataSets was the first step to the collection of data, providing access to primary submitter files and curated datasets. In the differential expression analysis of genes from these collected samples, we used the Galaxy server which is one of the broad-spectrum bioinformatics tools that suit various applications in the genomics research field. The DAVID bioinformatics resource system was used to convert the gene IDs of differentially expressed genes (DEGs) into gene symbols (Sherman et al. 2022), which is known for its functional annotation and enrichment analyses. WebGestalt, is a suite for functional enrichment analysis, was used to carry-out gene ontology and pathway enrichment analyses, focusing on the biological circumstances where the DEGs are expressed. (Wang et al. 2017).

The STRING database tool were used to identify and analyse protein-protein interactions among the filtered DEGs (Szklarczyk et al. 2021). For visualizing these interactions and networks, Cytoscape, a platform designed to handle large networks of interactions, was used (Shannon et al. 2003). Then, the Draw Venn Diagram tool was used to get list of unique and common genes among the samples.

To understand the gene expression patterns based on the biological pathways, the KEGG database (Kanehisa et al. 2023), which offers high-level functions and uses of biological systems from molecular-level data was used, PubChem (Kim et al. 2023) an open chemistry database that contains comprehensive information on various chemical compounds, was used to get the 3D structure of the catechin molecule.

Protein Data Bank (PDB) (Berman 2000) were used to obtain 3D structure data for the target genes. Finally, PyRx, a virtual screening software (Dallakyan and Olson 2015), was used to perform molecular docking of the target proteins with the catechin molecule. This approach was used in identifying the genetic biomarkers that are responsible for cellular senescence in mesenchymal stem cells derived from adipose tissue, umbilical cord, and dental pulp.

RESULTS

SAMPLE COLLECTION

The study collected samples of mesenchymal stem cells derived from adipose tissue, dental pulp, and umbilical cord, focusing on cellular senescence. Using the GEO DataSets database, 47 samples within passages 3-12 were gathered: 21 control samples (passages 3-6) and 26 case samples (passages 7-12) (Table 1). Specifically, the GSE for umbilical cord samples was GSE233634, for adipose tissue samples were GSE142761, GSE138879, GSE77284, GSE105077, and for dental pulp samples were GSE165627, GSE161688, GSE185751.

Table 1: Sample count taken from each tissue as case and control

Sample count	Umbilical cord	Adipose tissue	Dental pulp
Case	6	12	8
Control	6	10	5

DATA UPLOAD

Segregated data samples were uploaded to the Galaxy India server, converting sequence data into fastqsanger format. Quality control was performed using Trimmomatic and FastQC, ensuring high-quality reads.

SEQUENCE ALIGNMENT AND READ COUNTS

Using HISAT2, sequences were aligned to the human genome (hg38), producing BAM files. FeatureCounts and DESeq2 were then used to generate normalized transcript counts and perform differential expression analysis.

DIFFERENTIALLY EXPRESSED GENES

DESeq2 analysis produced dispersion plots, confirming good fit and dispersion rates for the model. MA-plots highlighted genes passing the significance threshold (adjusted p-value < 0.1), showing up-regulated and down-regulated genes across the samples (Fig. 1).

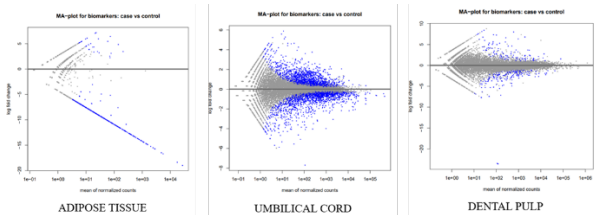


Fig. 1 The MA-plot graphically displays the up-regulated and down-regulated genes of the stem cell samples

GENE ONTOLOGY AND PATHWAY ANALYSIS

WebGestalt was used for gene ontology and pathway enrichment analysis. Bar charts depicted the distribution of genes across biological processes, cellular components, and molecular functions. Pathway analysis focused on genes significantly associated with cellular senescence.

GENE INTERACTION AND NETWORK ANALYSIS

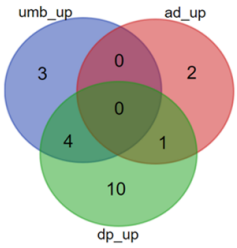
STRING database provided interaction networks for up-regulated and down-regulated genes, then visualized with Cytoscape. CytoHubba identified top 25 hub genes based on centrality measures (betweenness, MCC, degree, clustering coefficient).

VENN DIAGRAMS AND INTERPRETATION

Combining up-regulated and down-regulated genes from adipose tissue, dental pulp, and umbilical cord samples, we identified 20 up-regulated and 10 down-regulated genes directly involved in the cellular senescence pathway, with the help of KEGG database.

Venn diagrams identified unique and common genes among the samples. For cellular senescence pathways, up-regulated genes included PIK3R2, HRAS, and PIK3R1, while down-regulated genes included TGFBR1, EIF4EBP1, and CDKN2B (Fig. 2 and Fig. 3). This depicts that there is not much of a significant intersection among the samples in terms of their gene expression. On the other hand, the sample possess unique gene expression among the samples.

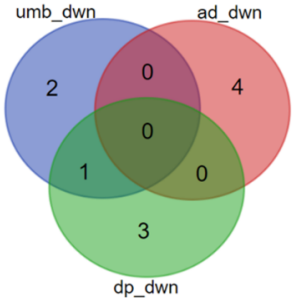
List names	number of elements	number of unique elements
ad_up	3	3
dp_up	15	15
umb_up	7	7
Overall number of unique elements		20



Names	total	elements
dp_up umb_up	4	PIK3R2 HRAS PIK3R1 PIK3CB
ad_up dp_up	1	CCND1
umb_up	3	PIK3CD CCND3 PIK3R3
ad_up	2	IGFBP3 SERPINE1
dp_up	10	NRAS CAPN2 MAPK3 PIK3CA HLA-B CCND2 AKT1 ATM MAPKAPK2 MAPK1

Fig. 2 Venn diagram with the list of common and unique up-regulated genes among all 3 set of samples (ad_up – Adipose tissue Up regulated

List names	number of elements	number of unique elements
ad_dwn	4	4
dp_dwn	4	4
umb_dwn	3	3
Overall number of unique elements		10



dp_dwn umb_dwn	1	TGFBR1
umb_dwn	2	EIF4EBP1 CDKN2B
ad_dwn	4	CALML6 MAPKAPK2 PPP1CA MAPK1
dp_dwn	3	IL6 NFATC1 FOXO1

Fig. 3 venn diagram representation along with the list of common and unique down-regulated genes among the 3 set of samples

PATHWAY-GENE EXPRESSION INTERPRETATION

Genes were analyzed for their roles in cellular senescence and related pathways (FOXO, mTOR, PI3K-AKT, cell cycle, P53). For example, up-regulated genes like PIK3R2 and HRAS were involved in multiple pathways, contributing to senescence (Fig. 4).

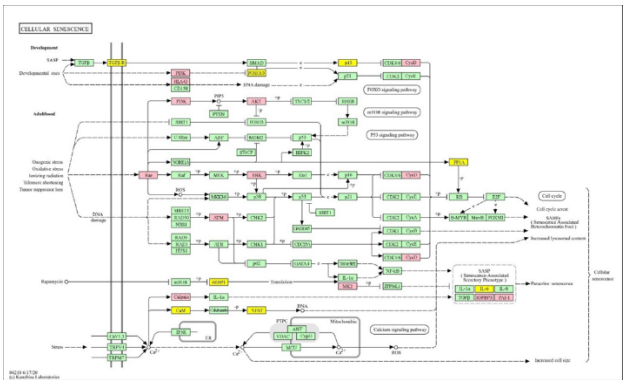


Fig. 4 The cellular senescence pathway highlighting the expressed genes

(The up-regulated genes are color-coded pink and the down-regulated genes are color-coded yellow. This representation is obtained by KEGG database)

UMB: Up-regulation in PI3K/AKT and RAS/MAPK pathways promotes increased cellular activity, DNA damage, and senescence.

AD: Up-regulation in IGF signaling and SASP leads to apoptosis, cell cycle arrest, and inflammation, contributing to senescence.

DP: Enhanced stress responses, immune activity, and cell proliferation, promoting senescence.

Understanding these changes provides insights into the molecular mechanisms driving senescence and potential therapeutic targets.

RETRIEVAL OF TARGET PROTEIN AND LIGAND STRUCTURES

Protein structures were retrieved from the Protein Data Bank, and catechin molecule structure was obtained from PubChem. Molecular docking was performed using PyRx, identifying binding affinities.

MOLECULAR DOCKING

We obtained the 3D structures of cellular senescence target proteins from the Protein Data Bank. Protein preparation included adding polar hydrogens, assigning partial charges, and removing water molecules. The catechin molecule's 3D structure was prepared in PDBQT format and docked using AutoDock Vina.

Initially, docking results showed high binding affinity scores ranging from -8 to -9 against HRAS, NRAS, MAPK1, MAPK3, PIK3CA, PIK3CD against catechin. Then, docking results showed high binding affinities for catechin with proteins such as NRAS (-8.5), comparing the docking results obtained by replacing the catechin with the co-crystallized ligand, mentioning the active sites (Table 2). These findings suggest potential interactions that may influence cellular senescence, with further in-vitro analysis required to confirm these results.

Table 2: Tabular representation of the highest binding affinity scores of the target proteins at the active sites against their co-crystallized ligands Vs the catechin molecule after molecular docking

Phytochemical	*Protein*	*Highest binding affinity with their co-crystallized ligand*	*Binding affinity with catechin molecule*
Catechin	NRAS	-7.2	-8.5
	HRAS	-9.7	-8.9
	MAPK1	-11.5	-8.4
	MAPK3	-9.9	-8.0
	PIK3CA	-9.0	-9.0
	PIK3CD	-10.1	-7.6

5. DISCUSSION

The present study aims to identify reliable biomarkers responsible for senescence in three types of mesenchymal stem cells (MSCs): adipose tissue-derived, dental pulp-derived, and umbilical cord-derived MSCs (Kim et al., 2022). The research involved collecting data, preprocessing it, and conducting quality control before performing sequence alignment with the human reference genome (hg38) using the HISAT2 tool. Gene counts were obtained through the featureCounts tool, and differential expression analysis of genes was conducted using the DESEQ2 tool.

Gene expression analysis revealed both upregulated and downregulated genes, which were further examined through gene set enrichment analysis, network analysis, and pathway enrichment analysis. The STRING database was utilized to analyze gene interactions, and Cytoscape software, along with the CytoHUBBA plugin, was employed to measure and rank the gene networks and hub genes identification using maximal clique centrality, betweenness centrality, degree of centrality, and clustering coefficient measures(Mengyan et al. 2022).

Venn diagrams were used to explore unique and common genes among the upregulated and downregulated genes in all three MSC samples. Pathway analysis identified 20 upregulated and 10 downregulated genes directly involved in the cellular senescence pathway.

In adipose tissue (AD), upregulated genes like IGFBP3 and SERPINE1 were found to modulate IGF signaling and regulate apoptosis and the senescence-associated secretory phenotype (SASP), contributing to senescence. In umbilical cord (UMB) MSCs, genes such as PIK3CD, PIK3R3, and CCND3, involved in the PI3K/AKT signaling pathway, enhance cell proliferation, growth, and survival, leading to increased cellular stress and senescence. Dental pulp (DP) MSCs exhibited upregulated genes like NRAS, CAPN2, and MAPK3, involved in RAS/MAPK and PI3K/AKT signaling, cell cycle regulation, DNA repair, and immune response, promoting enhanced cell proliferation, DNA damage, and immune responses, thus leading to senescence. Downregulated genes also play a role in senescence. In UMB, genes like EIF4EBP1 and CDKN2B, that control protein synthesis and cell cycle were downregulated,

resulting in the increased protein synthesis and absence of cell cycle control, serving as a senescence factor. In AD downregulated genes such as CALML6 and MAPKAPK2, which are involved in calcium signalling and stress response, were pointed-out, causing the disabled stress responses and increased cellular damage, which promoting senescence. In DP, downregulation of genes like IL6, NFATC1, and FOXO1, are key genes for immune response, calcium signalling, and stress response, results in reduced immune function, increased oxidative stress, and reduced DNA repair capacity, thus developing senescence.

The possible interaction of catechin, a natural product, with NRAS, one of the target proteins in cellular senescence, was predicted by the molecular docking experiment, the study that first involved the preparation of the 3D structures of the target proteins and all that were done to dock the catechin on them. NRAS was notably the most favourable primary responder as far as the binding of catechin was concerned, thus it is possibly a key player in the control of aging amid MSCs (24,25). This finding indicates that catechin could be an effective compound for further in-vitro studies to validate its therapeutic potential in mitigating cellular senescence.

The study provides talks about the molecular mechanisms driving cellular senescence in different MSC types and identifies potential therapeutic targets to encounter cellular senescence and improve tissue health. Further in-vitro validation of these findings could be helpful for developing anti-senescence therapies.

Statements & declarations

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Competing Interest:

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shalini U and Alan Punnoose. M. The Manuscript was supervised by Dicky John Davis G. Lavanya Prabhakar contributed to the final draft of manuscript for publication. All authors read and approved the final manuscript.

Data Availability:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

Sri Ramachandra institute of higher education and research Ethics committee has confirmed that no ethics approval is required.

Competing interest

Authors have no competing interest to declare..

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