



# RNA-seq Analysis of Senescent Mesenchymal Stem Cells

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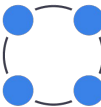
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# Introduction

Main directions of group on Genetics of stem cell differentiation and senescence

Application of human endometrial mesenchymal stem cells in regenerative medicine

Investigating the stress impact on cell cycle of human endometrial mesenchymal stem cells



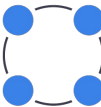
# Introduction

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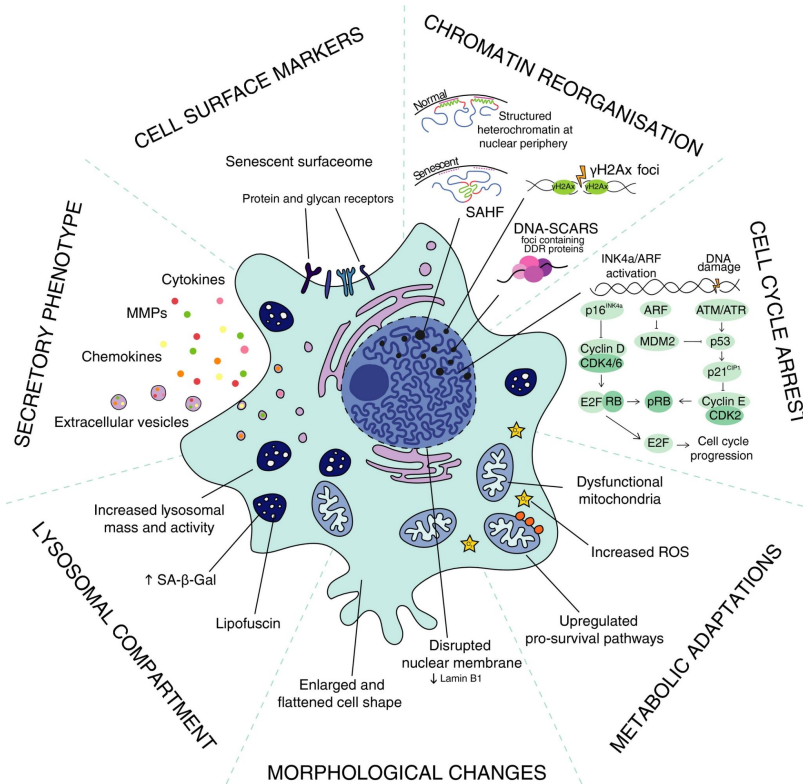
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**Problem of replicative senescence during cultivation!**



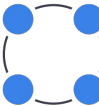
# Introduction



Cellular senescence is characterized by several hallmarks, including:

- stable cell cycle arrest mediated by proteins like p16INK4a/Rb and p21CIP1/p53
- chromatin alteration and reorganization
- macromolecular damage and metabolic changes
- resistance to apoptosis
- increased lysosomal compartment,
- secretion of SASP.

While these markers are commonly associated with senescence, they are not always present or essential, except for the cell cycle arrest.





# Goal

Observe changes in transcriptome profile of endometrial mesenchymal stem cells (eMSC) through cultivation until replicative senescence.

## Objectives:

- Align transcriptomes of high-proliferative eMSC (Young), eMSC after one month of cultivation (Medium), and replicative senescent eMSC after two months of cultivation (Senescent)
- Cluster sample
- Analyze DEG of Medium and Senescent groups against Young group
- Find enrichment pathways and pathways categories for Medium and Senescent groups
- Compare pathways between Medium and Senescent groups



# Methods

fastq files

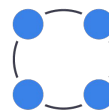
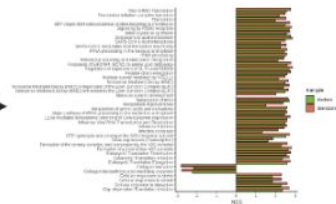
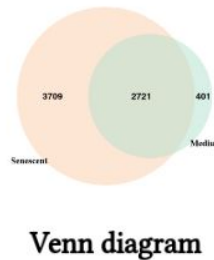
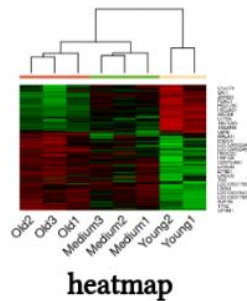
hisat2

featureCounts

Deseq2

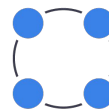
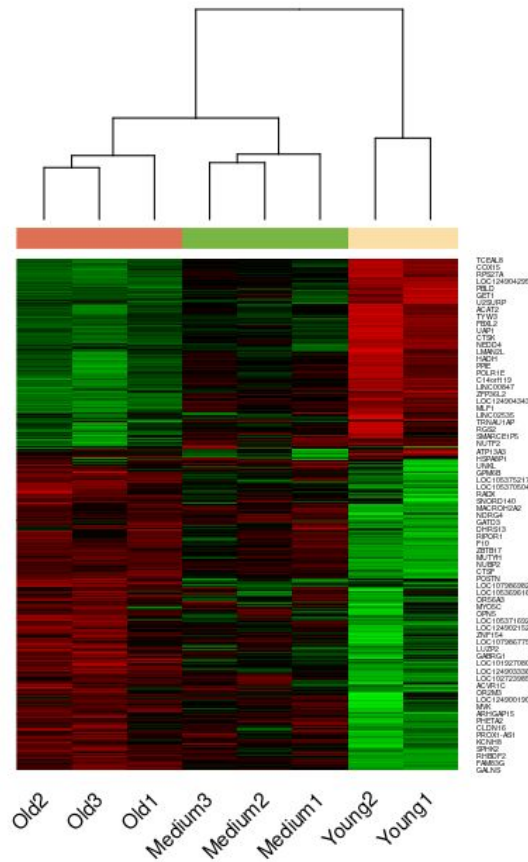
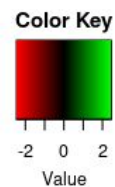
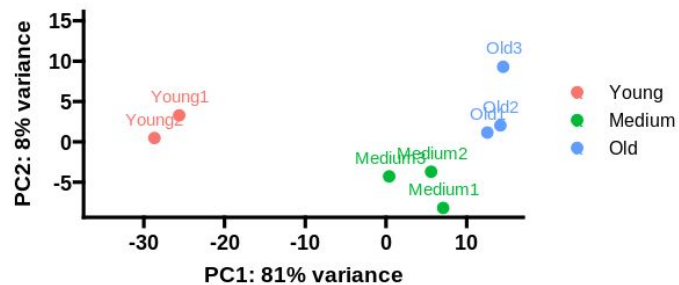
ReactomePA

enriched pathways



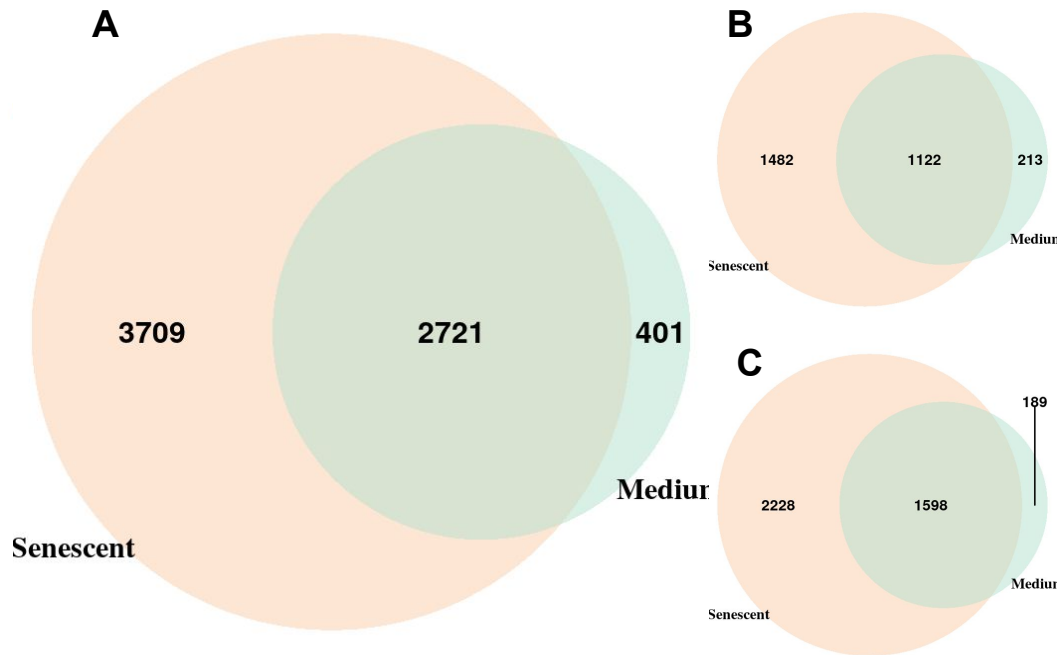
# Results

After one month of cultivation cellular transcriptome became closer to replicative senescent cells than to high-proliferative ones

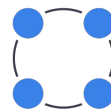




# Results

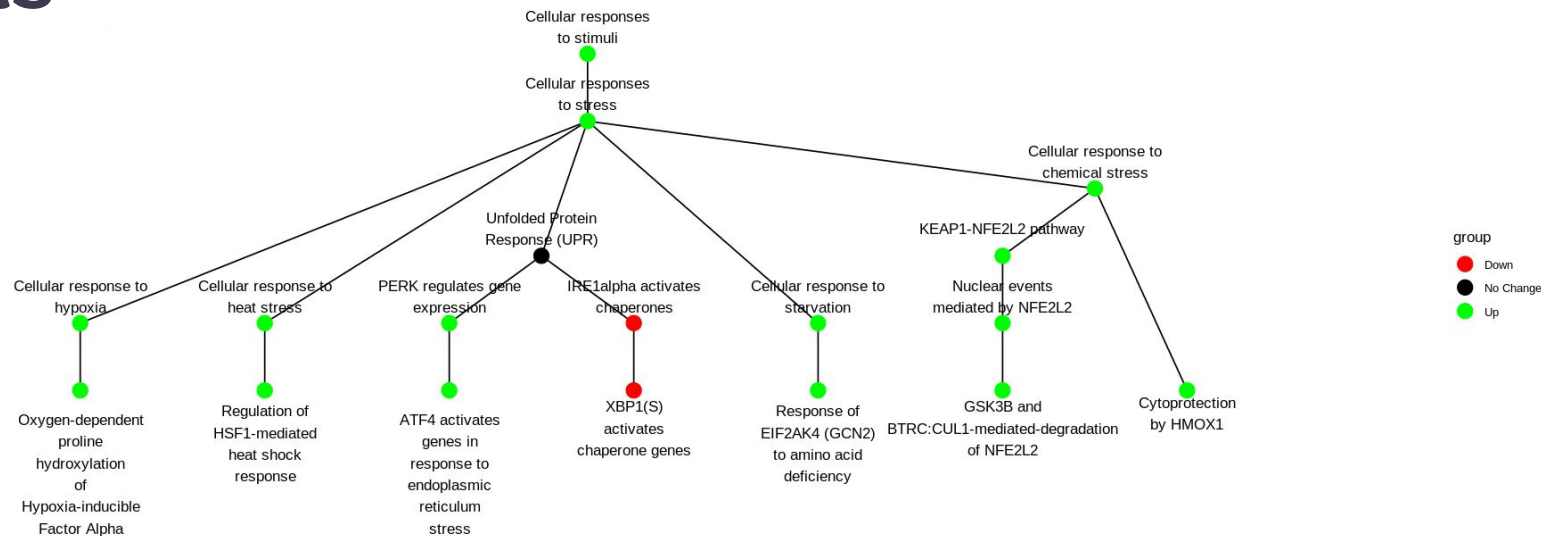


Venn diagrams show the more than two-fold significant genes in Medium and Senescent samples against control. (A) Up- and Down-regulated genes. (B) Up-regulated genes. (C) Down-regulated genes. The majority of significant genes for Medium overlaps with Senescent significant genes.

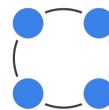




# Results



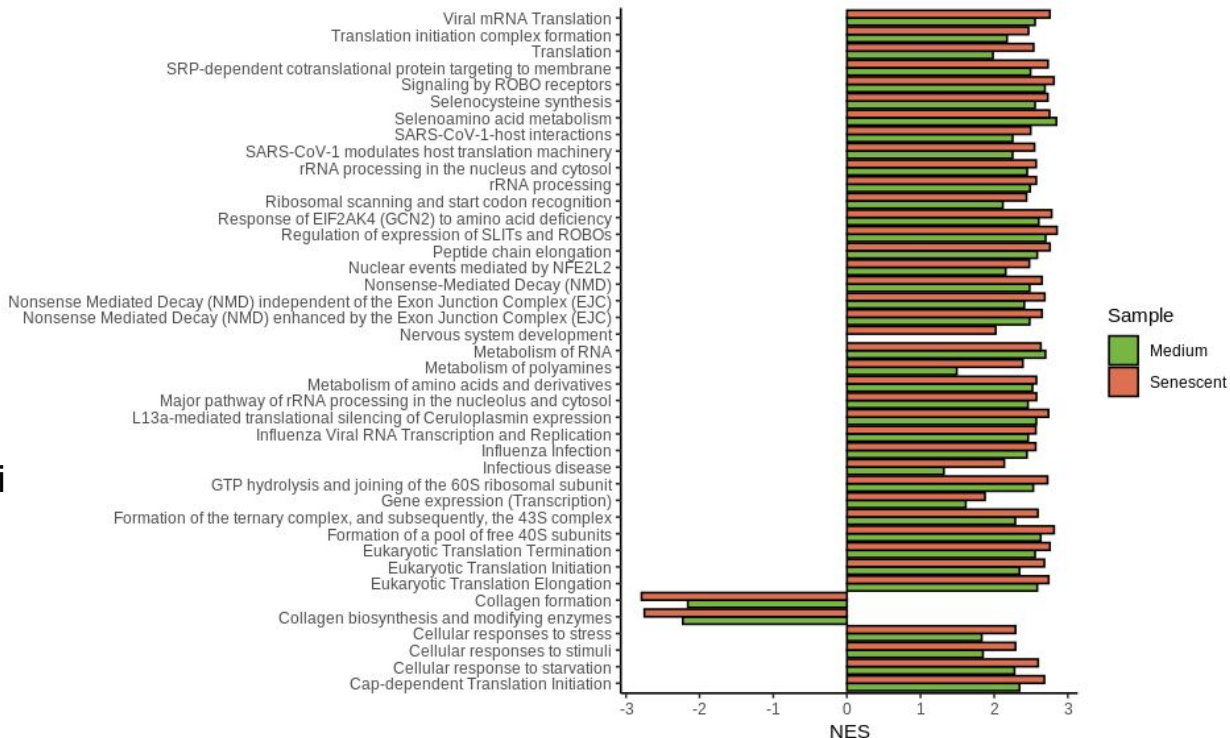
Example of pathway tree for *Cellular responses to stimuli* Category of Senescent sample. Different colors represent down-regulation (red), up-regulation (green), and no change in regulation (black) of the pathway.



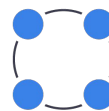
# Results

The most enriched pathway categories:

- Cellular responses to stimuli
- Metabolism of RNA
- Gene expression (Transcription)
- Extracellular matrix organization
- DNA Replication
- Developmental Biology
- Cell Cycle
- DNA Repair



The NES score for most significant pathways for Senescent and Medium pathways. Almost all pathways are more affected in Senescent sample.





# Future Plans

Our findings indicate that eMSCs show signs of senescence in their transcriptome after one month of cultivation (half way life), despite remaining proliferatively active. This analysis provides valuable insights for future experiments evaluating cellular senescence in eMSCs.

