Revealing common lncRNAs and gene signatures: computational analysis of public RNA-seq datasets in different *in vitro* senescence models

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Background and aims

Senescence is a **cellular state** characterized by many **molecular alterations**: permanent cell cycle arrest, senescence-associated secretory phenotype (SASP), increased β-galactosidase activity (SA-β-gal) and morphological changes[1,2]. Growing evidence suggest that **long non-coding RNAs (lncRNAs) play important roles in cellular senescence**[3-5] **and age-related diseases**[6-8] at multiple levels-transcriptional, post-transcriptional, translational, and post-translational. However, **their exact biological function remain largely unclear**[9].

The aim was the identify **differentially modulated genes/lncRNAs and pathways shared by** different type of human cells undergoing senescence, by utilizing **open access bulk RNAseq** datasets and computational analysis.

Bionformatics pipeline

RNAseq datasets were selected from the open access archive "Gene Expression Omnibus" (GEO) focusing on experiments regarding *in* ***vitro*** **models of replicative senescence (RS) and drug induced-senescence (Table 1),** which were supported by published articles[10-16]. The bioinformatics pipeline was developed in R [v4.2.2, 2022-10-31] and included the following steps:

- Downloading of preprocessed raw data matrix from GEO.
- Annotation of data matrix using the human Build GRCh.38.p13.
- Data filtering and normalization using R packages Limma [v.3.54.2] and EdgeR [v.3.40.2].
- Computation of differentially expressed genes and lncRNAs (DEGs) with an adjusted p-value < 0.05 and log2 fold-change > [0.58] using DESeq2[v.1.38.3].
- Pathway enrichments analysis using the gprofiler2 [v.0.2.3].

Results

Table 1: List of selected RNAseq datasets and number of up- and down-regulated genes and lncRNAs (DEGs)

Human fibroblast (HF) datasets	DEGs up⇧/down⇩	Mesenchymal stromal cells (MSC) datasets	DEGs up⇧/down⇩	Vascular smooth muscle cells (VSMC) datasets	DEGs up⇧ down⇩
GSE109700[10]	3255⇧/3247⇩	GSE98440[13]	360⇧/525⇩	GSE171663[16][RS]	230⇧/470⇩
GSE60340 [11]	1516⇧ /1645⇩	GSE59966 [14]	928⇧/798⇩	GSE171663[16][Dox1d]	1490⇧/1532⇩
GSE63577[12] [BJ]	900⇧/842⇩	GSE125632[15]	258⇧/579⇩	GSE171663[16][Dox21d]	328⇧/655⇩
GSE63577[12][HFF]	2961⇧/2316⇩				
GSE63577[12][IMR90]	1156⇧/1163⇩				
GSE63577[12][MRC5]	1308⇧/1542⇩				
GSE63577[12][WI38]	2534⇧/2226⇩				

Comparion of the pathway enrichment analysese based on the list of DEGs of each dataset depicted by bubble plots

Figure 1. Shared KEGGs terms in human fibroblast datasets

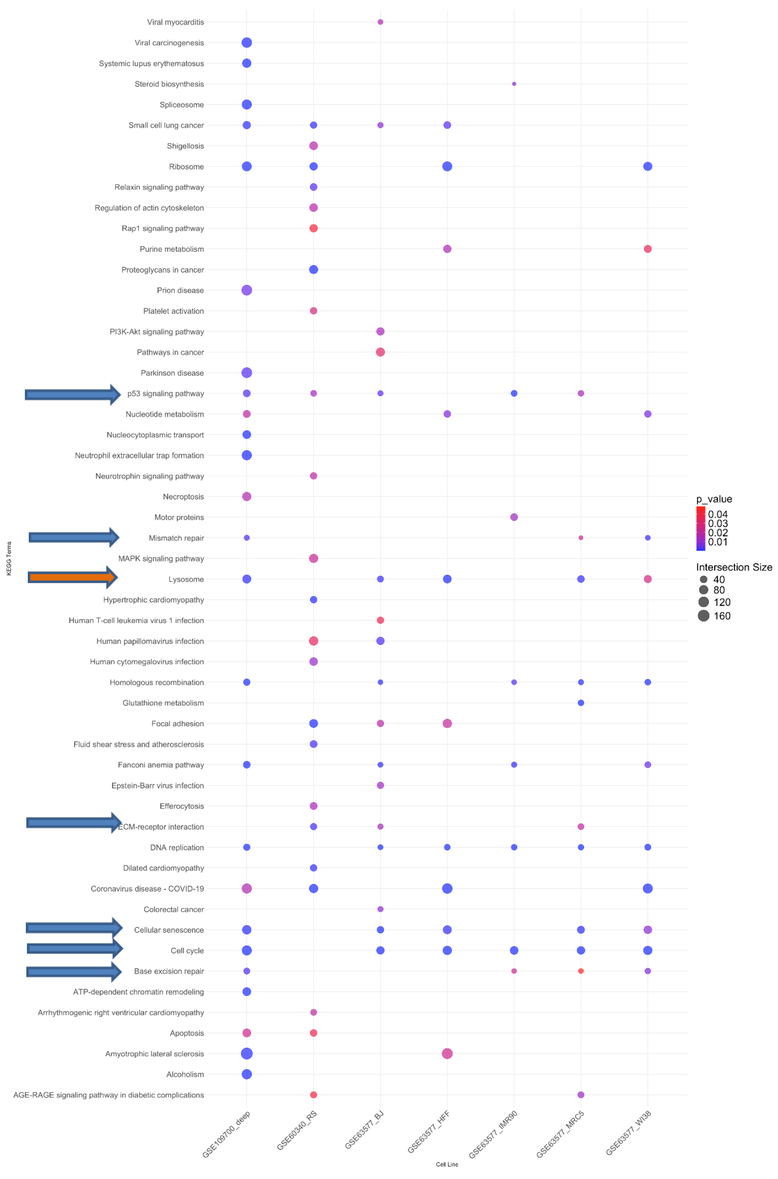


Figure 2. Shared KEGGs terms in mesenchymal stromal cells datasets

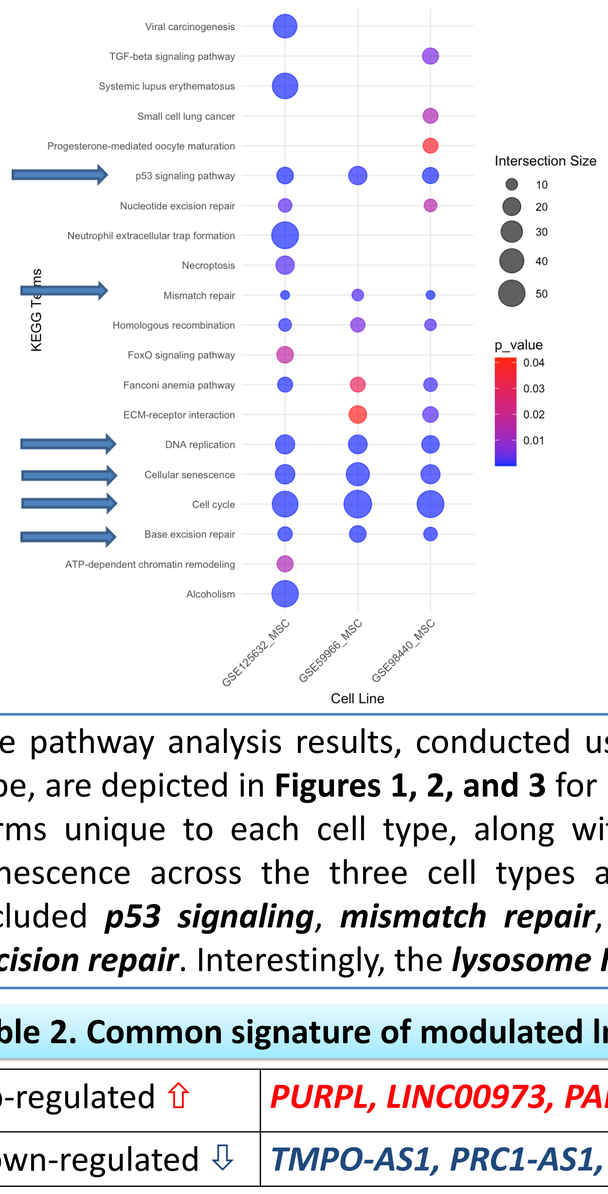
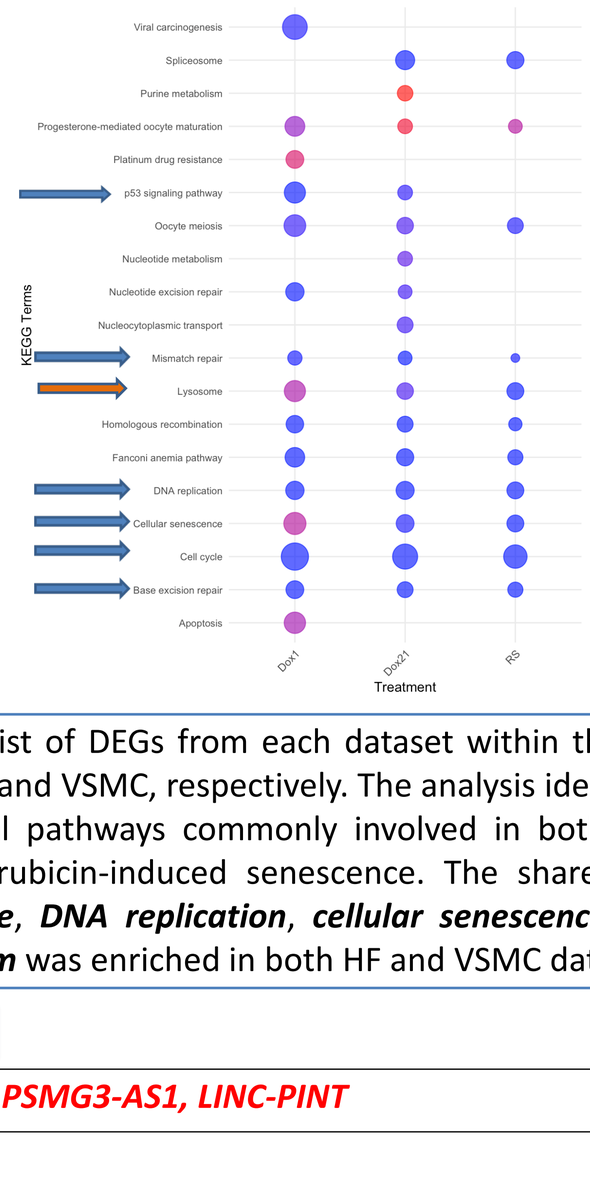


Figure 3. Shared KEGGs terms in vascular smooth muscle cells datasets



The pathway analysis results, conducted using the list of DEGs from each dataset within the same cell type, are depicted in **Figures 1, 2, and 3** for HF, MSC, and VSMC, respectively. The analysis identified KEGG terms unique to each cell type, along with several pathways commonly involved in both replicative senescence across the three cell types and Doxorubicin-induced senescence. The shared pathways included ***p53 signaling***, ***mismatch repair***, ***cell cycle***, ***DNA replication***, ***cellular senescence***, and ***base excision repair***. Interestingly, the ***lysosome KEGG term*** was enriched in both HF and VSMC datasets.

Table 2. Common signature of modulated lncRNAs

Up-regulated ⇧	***PURPL, LINC00973, PAPPA-AS1, PSMG3-AS1, LINC-PINT***
Down-regulated ⇩	***TMPO-AS1, PRC1-AS1, DLEU2***

Conclusions and future plans

In summary, we identified **distinct signatures of modulated protein-coding genes**, leading to both specific and **commonly shared** pathways. These pathways further validated the **molecular signatures of the senescence models**. These pathways further corroborated the molecular signatures characteristic of the senescence models. As extensively documented, key processes such as cell cycle regulation, DNA damage repair, and p53 signaling are well-established hallmarks of senescence. Additionally, the analysis of lncRNAs confirmed **the upregulation of PURPL and uncovered novel lncRNAs** (**see Table 2**). Future analysis will focus on exploring the regulatory networks of these modulated lncRNAs across various cell types during senescence.

References

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