Co-expression network for better understanding of cellular senescence

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Abstracts

Cellular senescence is a state of irreversible growth arrest derived from a variety of stress. Senescent cells were accumulated during aging and have been implicated in promoting a variety of age-related diseases. In this study, we collected and analyzed public RNA-seq data associated to senescence to identify the heterogeneous characteristics of senescence through weighted gene co-expression network analysis (WGCNA). Firstly, we selected public senescence RNA-seq data caused by four most well-known inducers: replicative exhaustion, DNA damage response, chemotherapy, and oncogene hyper-activation. Raw sequence files of five RNA-seq data sets, including 74 samples, were obtained from the Gene Expression Omnibus database. Secondly, after data preprocessing with the same analysis pipeline, 14,150 genes with low-expression genes removed were selected for WGCNA. After constructing a co-expressed network for senescence, we divided it into 51 modules. Among them, we found that the seven modules were highly correlated with cellular senescence, and especially among nine modules, six modules were significantly important based on gene ontology analysis. Lastly, when we analyzed the co-expression network for senescence based on HumanNet V3 functional gene network with genes from selected modules, we could identify 447 hub genes of the top 10% of high-connectivity within the modules. In conclusion, based on co-expression network analysis, we could identify new key candidate genes for understanding cellular senescence.

Introduction

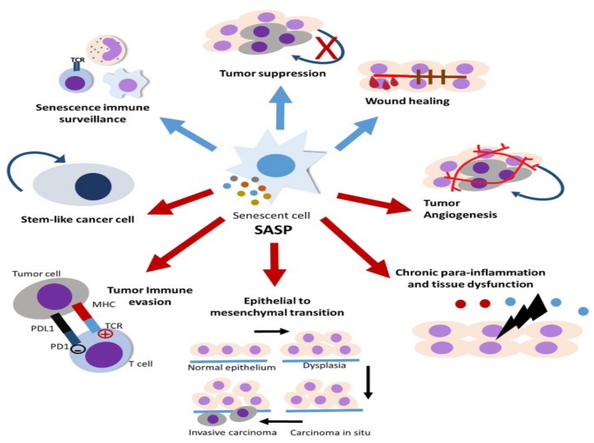


Figure 1. Cellular senescence effects

Cellular senescence is a stress response that elicits a permanent cell cycle arrest and triggers profound phenotypic changes such as the production of a bioactive secretome, referred to as the senescence-associated secretory phenotype (SASP). Acute senescence induction protects against cancer and limits fibrosis, but lingering senescent cells drive age-related disorders. Since senescence is a very heterogeneous and complex cellular phenotype, we constructed gene co-expression network through RNA-seq analysis to understand cellular senescence in detail. Therefore, we performed weighted gene co-expression network analysis (WGCNA). Finding cluster (modules) of highly correlated genes through WGCNA and by associating these modules with external sample traits (using eigengene* network methodology) that we were interested, we could find the most significant modules of senescence.

Eigengene*: eigengene is defined as the first principal component of a given module.

Materials & Methods

Table 1. Public RNA-seq datasets for cellular senescence

Public Data Set Reference and Accession ID	Organism	Cell-line	Platforms (Homo sapiens)	Cellular senescence Inducer	Total no. of samples	No. of cases	
						control	senescence
S. Marthandan et al. 2016 GSE63577	Homo sapiens	IMR90, WI-38	Illumina HiSeq 2000	Replicative exhaustion	12	6	6
Dikovskaya D et al. 2015 GSE70668	Homo sapiens	IMR90	Illumina NextSeq 500	Oncogene hyper-activation	6	3	3
Hoare M et al. 2016 Goncalves et al. 2021 GSE72407	Homo sapiens	IMR90	Illumina HiSeq 2000	Oncogene hyper-activation, Chemotherapy	24	8	16
Casella G et al. 2019 GSE130727	Homo sapiens	IMR90, WI-38	Illumina HiSeq 2500, Illumina HiSeq 4000	Replicative exhaustion, Oncogene hyper-activation, Chemotherapy, Ionizing radiation	26	12	14
Vizioli M et al. 2020 GSE132370	Homo sapiens	IMR90	Illumina NextSeq 500	Ionizing radiation	6	3	3

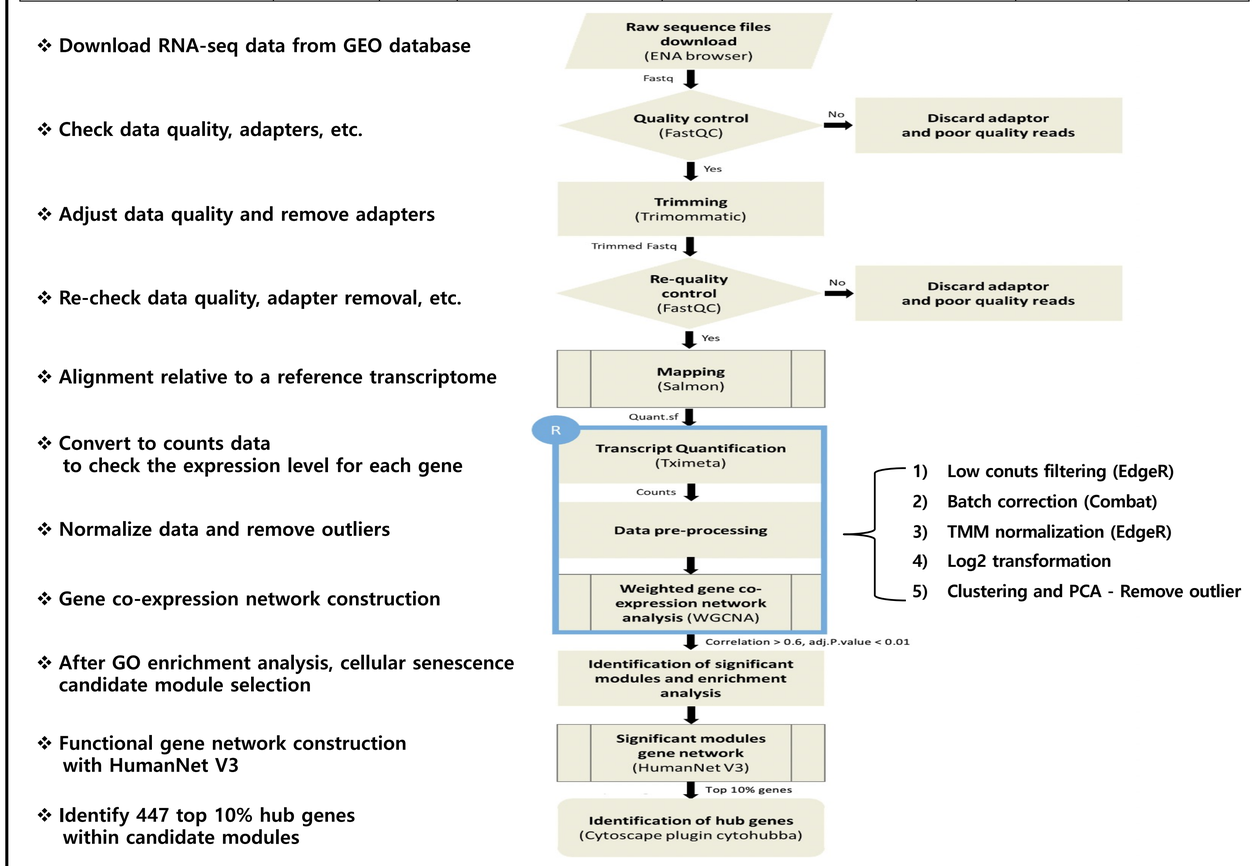


Figure 2. Weighted gene co-expression analysis pipeline for RNA seq data

Results

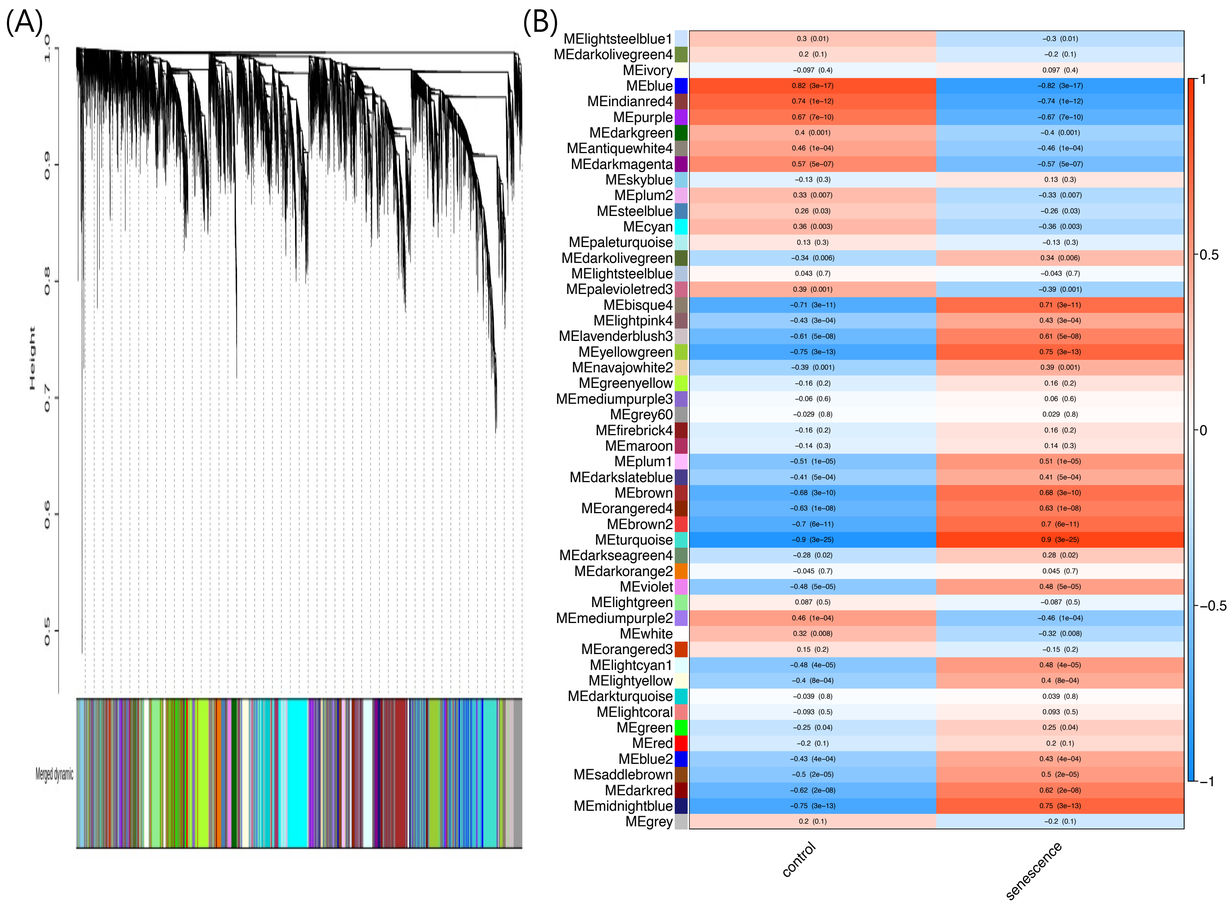
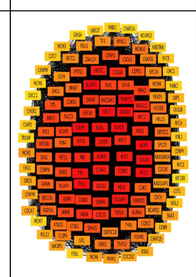
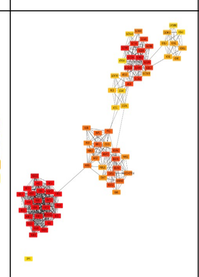
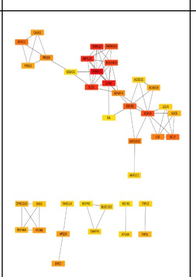
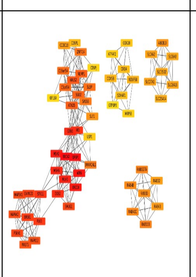
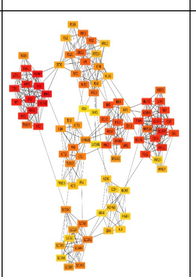
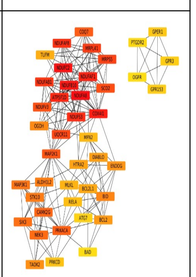


Figure 3. The cluster results by weighted gene co-expression network analysis (WGCNA)
(A) Gene cluster dendrogram and color coding of co-expression network modules. (B) Module-trait relationship. Heatmap of correlation between eigengene modules and control(left) or senescence(right).

Table 2. Gene ontology (GO) enrichment analysis in candidate modules on cellular senescence

Module	Cor. sene	Adjusted p value	No. of genes	Gene Ontology(MF, BP, CC)	Pathways(Wiki, Reactome, KEGG)
Turquoise	0.9	1.63E-23	1550	Cell cycle, DNA replication, cell division, DNA repair, DNA recombination, chromosome (telomeric region)	Cell cycle, DNA replication, DNA repair, cell cycle checkpoints, DNA IR-damage and cellular response via ATR
Yellowgreen	0.75	3.49E-12	864	Nucleosome assembly, chromatin assembly, organelle membrane, organelle subcompartment, glycerolipid metabolic process	Cellular responses to stress, SIRT1 negatively regulates rRNA expression, neutrophill extracellular trap formation, cellular senescence, signaling WNT and NOTCH, SASP
Midnightblue	0.75	3.49E-12	415	Indanol dehydrogenase activity, organelle, transcription factor TFTC complex	.
Bisque4	0.71	2.53E-10	625	Cellular response to stress(DNA damage stimulus etc.), nucleoplasm, organelle localization, enzyme binding	DNA repair pathway full network, diseases of mismatch repair (MMR), miR-517 relationship with ARCN1 and USP1
Brown	0.68	1.99E-09	1012	Cytoskeleton and organelle organization, nucleus, ion and enzyme binding	Focal adhesion, gene expression (transcription)
Lavenderblush3	0.61	2.23E-07	464	Mitochondrion organization, autophage, positive regulation of intracellular signal transduction, vesicle membrane, kinase binding	IL-3 and IL-6 signaling pathway, oxidative phosphorylation, TCA cycle and respiratory electron transport

Table 3. Functional hub gene sub-network in each candidate module.

Module	Turquoise	Yellowgreen	Midnightblue	Bisque4	Brown	Lavenderblush3
Functional hub gene network						
No. of node	139	78	38	58	91	43
No. of edge	7131	581	66	246	472	205

Conclusions

- **We identified six gene sets associated with cellular senescence through weighted gene co-expression network analysis (WGCNA).**
- **We found 447 genes as top 10% hub genes in the selected modules. Those genes could become candidate key biomarkers for cellular senescence.**
- **In further studies, we plan to validate that the identified hub genes are significant for cellular senescence.**

References

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Acknowledgement

It was supported by Korea Foundation for the Advancement of Science and Creativity (SBJ000037951) and the National Research Foundation of Korea (NRF-2018R1C1B5032617).