Identification of candidate pathways mediating the senotherapeutic effect of o-vanillin and RG-7112

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INTRODUCTION: Low back pain is a global health problem that is directly related to intervertebral disc (IVD) degeneration. Senotherapeutics are classified into two major groups: senolytics, which selectively kill senescent cells (SCs) and senomorphics, which attenuate the pathological senescence-associated secretory phenotype (SASP). Senotherapeutics (RG-7112 & o-Vanillin) target and remove SCs from IVDs and reduce SASP factors release improving tissue homeostasis. The objective of this study is to reveal the pathways mediating the senolytic and senomorphic effects of each compound and the combination treatment in cells from degenerating human IVDs.

METHODS: All procedures performed were approved by the ethical review board at McGill University (IRB # 2019-4896). Pellet cultures of cells from human degenerate IVDs were exposed to TLR-2/6 agonist Pam2CSK4 to further induce senescence. They were then treated with the senolytics o-Vanillin and RG7112 alone or combined. RNA was extracted, and sequencing was performed using Next-Generation Sequencer NovaSeq 6000 PE100 to identify differentially expressed genes (DEGs). Bioinformatics and differential expression analysis, including 3D RNA-seq[1-2] and gene set enrichment analysis (GSEA)[3-4], were used to explore the candidate genes and pathways. The most significantly differentially expressed genes and possible pathways will be validated using qRT-PCR using additional samples. Critical enzymes and proteins involved in pro-survival and anti-apoptotic mechanisms will be targeted using pathways inhibitors, such as LY3214996 (ERK), Wortmannin (PI3K), Lisaftoclax (Bcl-2 and Bcl-xl) and others to confirm further the specificity of each pathway.

RESULTS SECTION: The RNA-seq data had 4-factor groups and each had 4 biological replicates (16 samples in total). Limma R package was used for 3D expression comparison[5-6]. For DEGs, the Log_2 FC of gene abundance was calculated based on contrast groups, and the significance of expression changes was determined using t-test. P-values of multiple testing were adjusted with BH to correct the false discovery rate (FDR)[7]. A gene was significantly DE in a contrast group if it had adjusted p-value < 0.05 and Log_2 FC ≥ 0.5. Results showed that DEGs were identified both in single and combination treatments and suggested different pathways that mediate the effect of each senolytic and their combination. Notably, over 77, 127 and 143 differentially expressed genes, with reference to the controls, were identified in IVD cell pellets treated with o-vanillin, RG-7112, or the combination respectively (Figure 1 & 2). Gene Ontology, KEGG pathway, and GSEA analysis demonstrated significantly enriched signaling pathways, such as TNF, NOD, NOTCH, JAK-STAT for o-vanillin and p53, PI3K-AKT, RAS for RG-7112. Importantly, new signaling pathways (TGFβ, WNT-β catenin, INFγ) were enriched in the combination group.

DISCUSSION: A comprehensive understanding of the mechanism of senotherapeutic is the key to improving their outcomes. Currently, the mode of action of the two senotherapeutics o-vanillin and RG-7112 remains unclear. Hence, we identified the potential pathways for each compound and their combination in human IVD cells. Identifying the mechanistic action of senotherapies, senomorphic or senolytic is challenging, and is often dependent on the heterogeneous transcriptional, metabolic, and SASP profiles of SCs and the concentration of the senotherapeutic. Thus, several pathways were identified suggesting a dual role (senolytic and senomorphic) for o-vanillin and RG-7112 that may target various senescence-mediating pathways or exhibit specificity towards specific sub-populations of SCs. The senotherapeutic effect improvement observed in the combination treatment[8] can be attributed to the new specific pathways (TGFβ, WNT-β catenin, INFγ) not activated following the treatment with the single compounds. However, there are still some limitations and shortages in our research. First, the sample size retrieved was small. Second, the results were lack of experimental validations in vivo. Despite these shortcomings, the preliminary study can still provide very meaningful and constructive findings.

SIGNIFICANCE/CLINICAL RELEVANCE: The development of senotherapeutics is expanding with more compounds and drug combinations being identified and tested in clinical trials. These findings promote the understanding of the molecular mechanism of two senotherapeutics (o-vanillin and RG-7112) as promising therapeutic targets for treating IVD-related low back pain

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IMAGES AND TABLES:

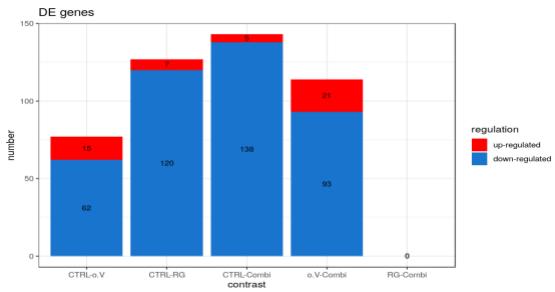

Figure 1. Number of up and down-regulated differentially expressed genes following the treatment with a single or a combination of o-vanillin and RG-7112. The numbers are calculated based on positive or negative signs on the Log2 FC of DE genes and an adjusted p-value < 0.05.

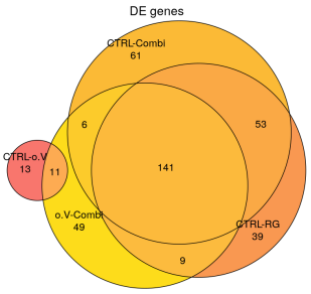

Figure 2. Gene co-expression. Venn diagram presents the number of significant DE genes that are uniquely expressed within each contrast group, with the overlapping regions showing the number of genes that are expressed in two or more samples.