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Network-Based Analysis of Rutin-Induced Gene Expression Changes in Human Senescent Stromal Cells

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ABSTRACT

Rutin, a lipophilic flavonoid of natural origin, occurs in various vegetables, citrus fruits, and drinks. The purpose of this research is to examine the metabolic routes of rutin within human senescent stromal cells. Datasets were obtained from the Gene Expression Omnibus (GEO) repository and initially processed with GEO2R to detect significantly differentially expressed genes (DEGs). These significant DEGs underwent protein-protein interaction (PPI) network evaluation to pinpoint hub genes. Hub genes were further refined through directed PPI analysis to isolate the most crucial DEGs. Visualization of the data involved volcano plots, Uniform Manifold Approximation and Projection (UMAP) plots, and Venn diagrams. From an analysis of 9124 significant DEGs, 33 upregulated and 61 downregulated hub genes were identified. Subsequent directed PPI analysis highlighted IL1B, ICAM1, CCL2, EGF, CXCL8, PTGS2, CAMK2B, CCN2, VCAM1, ELN, CXCL12, BGN, and TLR4 as the key hub genes. The genes IL1B, CCL2, GNAO1, ICAM1, EGF, and CXCL8 were identified as primary regulators modulated by rutin, while PTGS2 and CAMK2B stood out as the genes most strongly regulated. These results suggest notable positive impacts of rutin on the performance of the treated cells. Such impacts support the prospects of rutin as a potential plant-based therapeutic agent. Additional studies are, however, essential to mitigate possible adverse effects.

Keywords: Gene, Hub, Human, Rutin

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Introduction

Aging may be described as a gradual deterioration in organ performance coupled with diminished capacity to react to external cues. Efforts have been made by researchers to influence this process. Alterations in genomics and epigenetics [1], impaired mitochondrial activity [2], and various other disruptions can upset physiological balance, paving the way for age-linked pathologies. Cellular senescence represents a core feature of organ aging. It plays a role in the development of neurodegenerative disorders, cardiovascular conditions, and numerous cancers [3]. The contribution of senescent cells to a range of age-related pathologies is emphasized by approaches that target them to reduce many associated manifestations [4]. Senotherapeutics employ drugs to specifically act on senescent cells and are divided into two groups: senolytics, which trigger programmed cell death, and senomorphics, which inhibit the pro-inflammatory senescence-associated secretory phenotype (SASP) [5].
Senomorphics enable strategies to lessen the negative consequences of SASP without removing the cells entirely; this action mirrors that seen with compounds like aspirin, metformin, and resveratrol [6]. Various naturally occurring substances, particularly flavonoids and polyphenols, show strong antioxidant and anti-inflammatory properties capable of dampening inflammatory SASP. Kaempferol, for example, restrains SASP in human fibroblasts via lowered H2O2 generation, thus reducing the impact of senescence [7]. Rutin, a flavonoid derived from natural sources [8], is commonly present in vegetables and citrus fruits. Its ability to neutralize superoxide and peroxyl radicals confers valuable antioxidant advantages [9]. Extracts of Ginkgo biloba containing rutin have

been noted to lower extracellular amyloid beta deposits in Alzheimer's disease [10]. The capacity of rutin to contribute to anti-aging mechanisms, including its possible senomorphic effects and SASP inhibition, continues to be explored. In work by Lee *et al*., rutin was shown to improve skin elasticity and reduce wrinkles in skin fibroblasts, with increased expression of collagen I and A1 alongside reduced metalloproteinase levels [11].
Rutin is known to decrease the production of reactive oxygen species (ROS) triggered by UV exposure and to boost the function of superoxide dismutase (SOD), thioredoxin reductase, and glutathione peroxidase in plasma. Additionally, it helps maintain levels of vitamins C and E in skin fibroblasts exposed to UV radiation [12]. Pairing ascorbic acid with rutin further elevates catalase and SOD activities [13]. The precise molecular pathways through which rutin influences aging are not yet fully elucidated. Determining central proteins and genes associated with cellular senescence, together with exploring their interactions within rutin-involved metabolic processes, could aid in developing approaches to retard or avert aging. Tools from genomics and bioinformatics, including network analysis, offer effective means to reveal genes and proteins linked to aging that respond to rutin [14]. Human senescent stromal cells (PSC27) are derived from normal prostate tissue and consist mainly of fibroblasts with a minor fraction of non-fibrillar elements. These PSC27 cells can acquire SASP characteristics after exposure to genotoxic chemotherapy or radiation [15, 16]. This investigation sought to highlight essential genes participating in metabolic pathways influenced by rutin in the human senescent stromal cell line PSC27.

Materials and Methods

Data collection
To investigate the impact of rutin on normal human primary prostate stromal cells, the dataset GSE190279 was retrieved from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE190279>). Samples GSM5719090-2 represent normal human primary prostate stromal cells exposed to 50 μg/mL bleomycin (BLEO) and 100 μM rutin to trigger senescence. Samples GSM5719087-9 serve as the control group, treated solely with 50 μg/mL BLEO.

Pre-evaluation analysis
The dataset was analysed using GEO2R to identify potential significant differentially expressed genes (DEGs). Significant DEGs were visualised through a volcano plot, while sample clustering was evaluated with a Uniform Manifold Approximation and Projection (UMAP) plot. The total count of significant DEGs was established using a Venn diagram.

PPI network analysis
Significant DEGs were separated into upregulated and downregulated groups and constructed into respective PPI networks using Cytoscape software version 3.7.2 [17]. Protein data were entered into the “protein query” section of the STRING database, and initial networks were built with undirected edges. Network topology was examined via the “Network Analyzer” tool in Cytoscape to detect central nodes. A threshold of mean + 2 standard deviations (SD) was used to select hub genes. Upregulated and downregulated hubs were then integrated into an action map incorporating directed edges for activation, inhibition, and expression using CluePedia version 1.5.7, applying the default kappa score [18]. The resulting directed PPI network was further examined to identify key hubs according to out-degree and in-degree centrality measures.

Statistical analysis
Differentially expressed genes were deemed significant at an adjusted p-value < 0.05. PPI networks were generated with a confidence score set to 0.2.

Results and Discussion

Volcano plot visualisation revealed numerous genes with significant dysregulation **(Figure 1).** Non-significant changes clustered centrally in the plot. UMAP analysis clearly distinguished treated samples from controls (**Figure 2)**. The Venn diagram indicated a total of 9124 significant DEGs **(Figure 3).** An upregulated PPI network comprising 521 nodes and 7114 edges was generated, with a degree cutoff of 84 applied to select 33 upregulated hubs. The downregulated PPI network consisted of 1182 nodes and 26511 edges, and a degree cutoff of 122

identified 61 hubs. Both upregulated and downregulated DEGs were combined into a single directed PPI network **(Figure 4).** Node arrangements according to out-degree and in-degree values are presented in **Figures 5 and 6.** Previous research has explored the protective role of rutin against oxidative stress induced by a high-cholesterol diet. The capacity of rutin to modify gene expression in treated samples has been examined. Findings suggest that rutin largely restores gene expression levels altered by a high-cholesterol diet to near-control values [19].

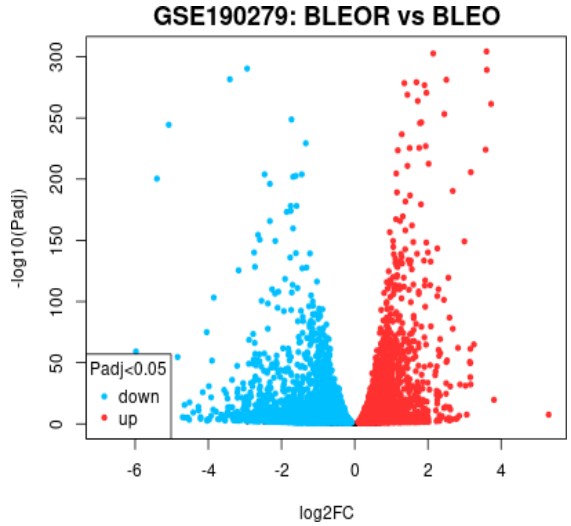

Figure 1. Volcano plot displaying genes that are significantly upregulated and downregulated among the differentially expressed genes (DEGs); BLEOR and BLEO indicate the cell groups treated with bleomycin combined with rutin and bleomycin alone, respectively [1].

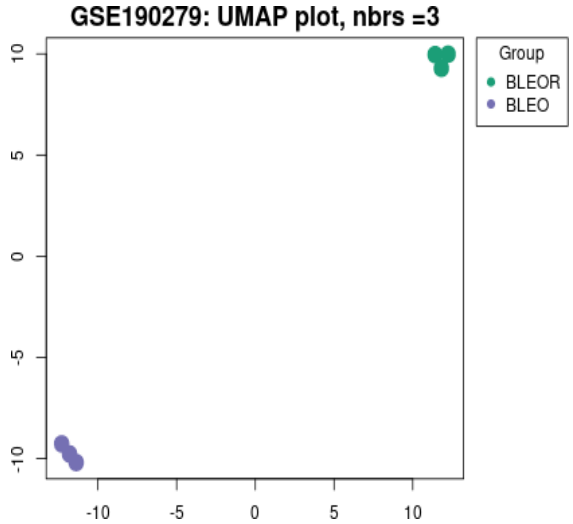

Figure 2. UMAP visualization of gene expression patterns in rutin-treated cells compared to control samples; BLEOR and BLEO indicate the cell groups treated with bleomycin combined with rutin and bleomycin alone, respectively; nbrs represents the count of neighboring points used to assess similarity [2].

Figure 3. Venn diagram illustrating the overlap in gene expression profiles between rutin-treated cells and controls; BLEOR and BLEO indicate the cell groups treated with bleomycin combined with rutin and bleomycin alone, respectively [3].

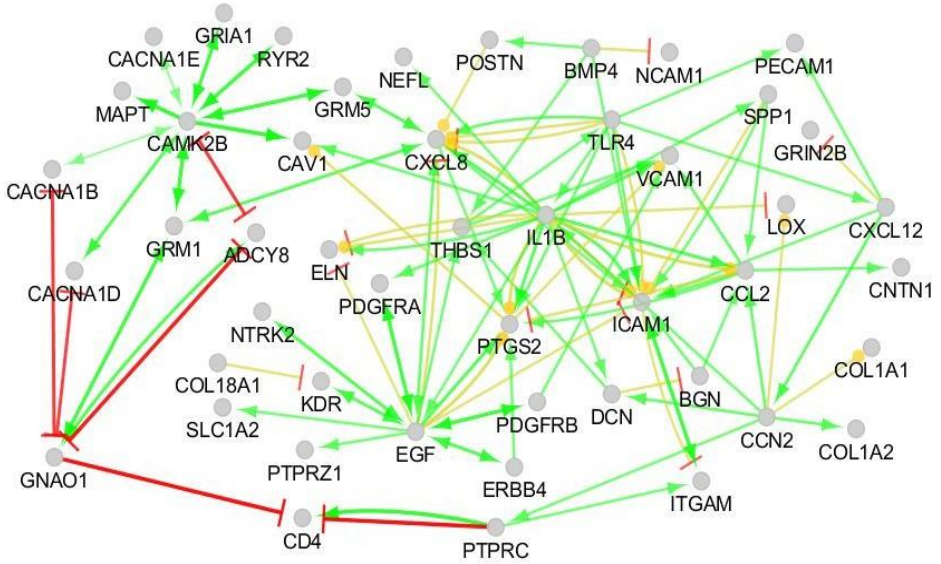

Figure 4. Directed protein-protein interaction (PPI) network featuring upregulated and downregulated hub genes; green edges denote activation, red edges denote inhibition, and yellow edges denote expression [4].

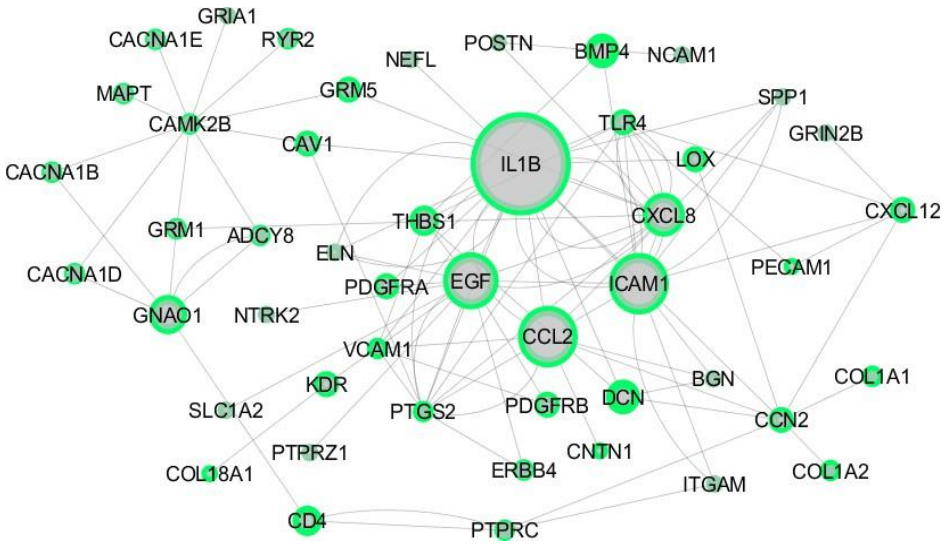

Figure 5. Directed protein-protein interaction (PPI) network featuring upregulated and downregulated hub genes; node positions are determined by out-degree values, with larger nodes corresponding to higher out-degree [5].

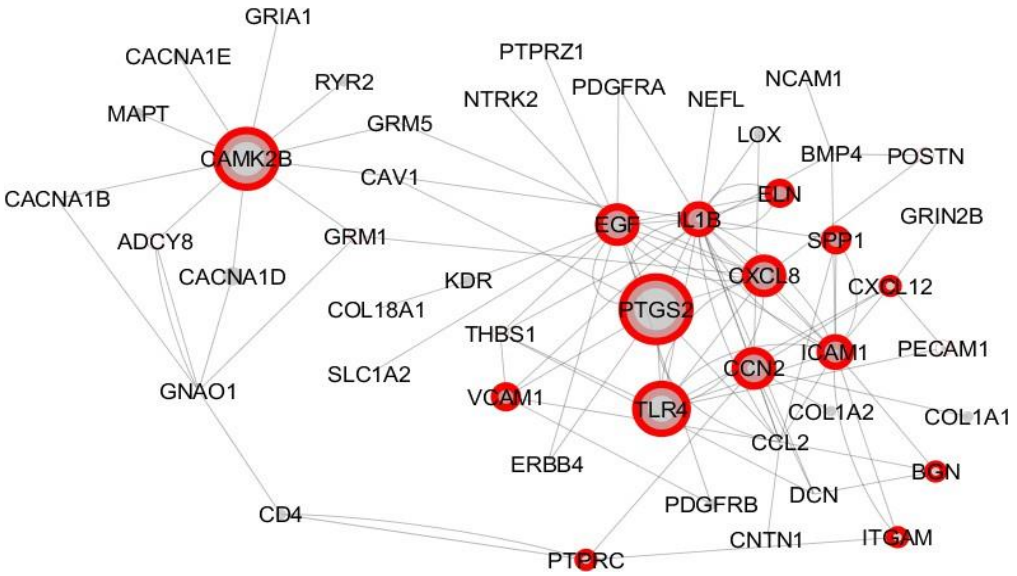

Figure 6. Directed protein-protein interaction (PPI) network featuring upregulated and downregulated hub genes; node positions are determined by in-degree values, with larger nodes corresponding to higher in-degree [6].

Figure 1 reveals that rutin administration led to significant dysregulation in a large number of genes [1]. The UMAP projection in **Figure 2** illustrates distinct clustering, with rutin-treated samples clearly segregated from the control group during the clustering process [2]. According to the Venn diagram in **Figure 3**, a total of 9124 differentially expressed genes (DEGs) with statistical significance were linked to rutin exposure [3].

Key upregulated and downregulated DEGs were pinpointed using protein-protein interaction (PPI) network evaluation. Hub genes are often utilized to uncover the molecular pathways involved in pathologies and therapeutic actions [20, 21]. A directed PPI network encompassing both upregulated and downregulated hub genes was built to select the most central ones. **Figure 4** demonstrates that roughly 50% of these hubs form part of the largest connected component, linked through activation, inhibition, and expression interactions [4].

Within directed graphs, out-degree measures the quantity of edges departing from a node [22]. Accordingly, in this hub-focused PPI network, a node's out-degree reflects how many direct neighbors it activates, inhibits, or influences via expression. Greater out-degree implies stronger dominance over adjacent nodes, marking it as a more powerful hub. **Figure 5** identifies IL1B, ICAM1, CCL2, EGF, CXCL8, and GNAO1 as the leading hubs when ranked by out-degree [5]. These appear to serve as dominant regulators in the overall network.

In-degree, conversely, counts the edges arriving at a node in a directed PPI setup [23]. **Figure 6** highlights PTGS2, CAMK2B, CCN2, ICAM1, CXCL8, EGF, and TLR4 as possessing the highest in-degree scores [6]. This indicates that these seven genes are primarily targets of regulation from their direct neighbors.

A notable direct link between IL1B and PTGS2 is visible in **Figure 4**, with IL1B topping the out-degree ranking and PTGS2 leading in in-degree. ICAM1, though downregulated, is prominent due to elevated scores in both metrics. Elevated serum concentrations of intercellular adhesion molecule 1 (ICAM1) have been observed in individuals with diabetes, alongside increased renal expression in diabetic animal models relative to normal subjects. Research indicates that ICAM1 drives the onset of diabetes and associated nephropathy [24].

CXCL8, also downregulated, ranks highly in both out-degree and in-degree. Known as C-X-C motif chemokine ligand 8 (CXCL8), it aids in eliminating pathogens but is implicated in harmful events like fibrosis, organ damage, tumor development, and new blood vessel formation [25]. Epidermal growth factor (EGF), the third highlighted downregulated hub, similarly shows strong values in both degrees and is tied to cancer development, potentially supporting the growth of cancerous cells [26].

As a downregulated hub with the highest out-degree, IL1B (interleukin 1 beta) has been linked to worsening major depression and altering treatment outcomes [27].

GNAO1 (G protein subunit alpha o1), marked by substantial out-degree, contributes to gastric cancer advancement; its elevated expression accelerates tumor growth and lowers patient survival rates [28]. CCL2 (CC motif chemokine ligand 2), an upregulated hub with high out-degree, exhibits heightened levels in tumor sites and could drive the advancement of various solid cancers [29].

PTGS2 (prostaglandin-endoperoxide synthase 2, or cyclooxygenase 2) downregulation has been noted in epilepsy cases during valproate therapy, as reported by Rawat C *et al*. [30]. Rutin induces similar downregulation here. CAMK2B (calmodulin-dependent protein kinase 2b), another downregulated hub, is vital for memory formation and neural adaptability in rodents. Evidence points to roles for both CAMK2A and CAMK2B in numerous brain-related and mental health disorders [31].

TLR4 (toll-like receptor 4), a downregulated hub, orchestrates swift responses to infections, but improper control can spark excessive immunity tied to conditions such as acute lung damage, septic shock, persistent harmful inflammation, malignancy, and autoimmunity [32]. The remaining upregulated hub, CCN2 (cellular communication network factor 2, also called connective tissue growth factor or CTGF), governs extracellular matrix buildup, cell sticking, programmed cell death, and growth. It has been connected to regional scarring, stem cell decisions, vessel formation, cancer growth, and inflammatory processes [33].

Although rutin offers benefits against diabetes, cancer, and neural decline, it also carries potential unwanted effects. Additional studies seem essential to confirm its safety for clinical use.

From the PPI evaluation in this work, rutin influences the activity of multiple genes tied to various dysfunctions and disorders.

Conclusion

Overall, the major molecular shifts caused by rutin in normal human primary prostate stromal cells include reduced expression of ICAM1, EGF, CXCL8, IL1B, GNAO1, PTGS2, CAMK2B, and TLR4, together with increased expression of CCL2 and CCN2. The genes IL1B, CCL2, GNAO1, ICAM1, EGF, and CXCL8 were found to act as controlling elements, while ICAM1, EGF, CXCL8, PTGS2, CAMK2B, CCN2, and TLR4 were determined to be the main targets under regulation. Notably, ICAM1, EGF, and CXCL8 belong to both controlling and controlled groups. Existing studies underscore the positive impacts of rutin on cellular health, such as safeguarding against diabetes, kidney complications from diabetes, and tumor development. At the same time, potential risks have been highlighted.

Therefore, expanded research is advised to better understand and harness the beneficial properties of rutin.

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Conflict of Interest: None

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