Identification of biological processes and potential inhibitors for aging skin

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Abstract

Aging is a critical risk factor for developing many diseases such as skin diseases. Aging skin is caused by the decline of regenerative potential and function of tissues. Here, we aim to discover the biological function and pathways of the skin from aged people. The GSE39170 dataset was originally created by the Illumina Genome Analyzer II (Homo sapiens). The biological pathways were analyzed by the Kyoto Encyclopedia of Genes and Genomes pathway (KEGG), Gene Ontology (GO), and Reactome. KEGG and GO results showed the extracellular matrices (ECMs) were mostly affected in the aging skin. Moreover, we discovered the top ten interacting proteins including FBN1, SPARC, THBS1, DCN, COL1A2, VCAN, LOX, SERPING1, FSTL1, and FBLN5 were involved in the aging skin. Further, we predicted several inhibitors that had the ability to block the aging process by L1000fwd analysis. Thus, this study provides further insights into the mechanism of aging skin.

Introduction

Aging is known as a lack of physiological integrity in the body[1]. A number of the processes associated with aging such as inflammation and cellular growth[2]. Besides the well characterized barrier function, the skin forms a barrier to defend against the infection[3].

Skin aging can be caused by internal and external reasons[4]. The extrinsic and intrinsic skin aging indicates various molecular mechanisms[5]. Several reasons can lead to skin aging such as mitochondrial DNA mutations and hormone dysfunctions[6]. Among them, gene mutation plays a central role in skin aging[7]. Kaisers W et al. found several aging-related alterations in the skin[8]. A study demonstrated the downregulation of mitochondrial function was the reason for the aging skin with the enhanced cytokine production[6]. Though there are numerous studies on aging related gene changes in the skin, the results and potential mechanism are still unclear.

In this study, we investigated gene expressions from the aging and young skins. We analyzed and identified several DEGs and the biological processes by using bioinformatics analysis. We analyzed the functional enrichment and protein-protein interaction for finding significantly changed genes. These findings could be crucial to prevent skin aging.

Methods

Data resources

The dataset GSE39170 was downloaded from the GEO database. It was produced by Illumina Genome Analyzer II (Homo sapiens), Dermatology Department, Stanford University. Bulk RNA-Seq analysis was performed using human skin of 5 women aged 50 years or more, and 5 young women aged 30 years or less.

Data acquisition and preprocessing

The dataset GSE39170 that contains young skin samples and aging skin samples was conducted by R script as described[9–14]. A classical t-test was used to identify DEGs with P< 0.01 as being statistically significant.

Gene functional analysis

The GO analysis and KEGG pathway enrichment analysis were performed by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (http://david.ncifcrf.gov/). P<0.05 was considered statistically significant.

Module analysis

The Molecular Complex Detection (MCODE) was used to construct the protein-protein interaction (PPI) networks[15]. The pathway enrichment analyses were performed by using Reactome, and P<.05 was used as the cutoff criterion.

Results

Identification of DEGs between the young and aging skin

To gain insights on aging skins, the modular transcriptional signature of skins from the aged women (> 50-year-old) was compared to that of the young controls (< 30-year-old). A total of 79 genes were identified to be differentially expressed with the threshold of P < 0.001. The top 10 up- and down-regulated genes for aging skins are listed in Table 1 and Figure 1. KEGG and GO analyses of DEGs between the young and aging skin

To further analyze the biological functions of the DEGs from the aging skins versus young controls, we performed KEGG and GO analysis. Our study showed the top ten enriched KEGG pathways including “PI3K-Akt signaling pathway”, “Human papillomavirus infection”, “Focal adhesion”, “Proteoglycans in cancer”, “Calcium signaling pathway”, “Regulation of actin cytoskeleton”, “Phospholipase D signaling pathway”, “Platelet activation”, “Protein digestion and absorption”, and “ECM–receptor interaction” (Figure 1).

We identified the top ten cellular components including “collagen–containing extracellular matrix”, “cell–cell junction”, “apical part of cell”, “focal adhesion”, “cell–substrate junction”, “cell leading edge”, “apical plasma membrane”, “sarcolemma”, “collagen trimer”, and “platelet alpha granule” (Figure 2). We then identified the top ten biological processes: “extracellular matrix organization”, “extracellular structure organization”, “cell-substrate adhesion”, “cell-cell adhesion via plasma-membrane adhesion molecules”, “regulation of actin filament-based process”, “regulation of actin cytoskeleton organization”, “homophilic

cell adhesion via plasma membrane adhesion molecules", "cell-matrix adhesion", "regulation of cell-substrate adhesion", and "collagen fibril organization" (Figure 2).

We identified the top ten molecular functions: "actin binding", "extracellular matrix structural constituent", "glycosaminoglycan binding", "actin filament binding", "integrin binding", "growth factor binding", "heparin binding", "collagen binding", "fibronectin binding", and "platelet-derived growth factor binding" (Figure 2).

PPI networks and Reactome

The PPI networks were created by using the String and the top two clusters were selected by using the Cytoscape (Figure 3). We set the criterion of combined score > 0.7 and constructed the PPI network by using the 73 nodes and 38 interactions. Among these nodes, the top ten genes with the highest scores are shown in Table 2. We identified several signaling pathways by using Reactome. We identified top ten signaling pathways including: "Extracellular matrix organization", "Elastic fibre formation", "Crosslinking of collagen fibrils", "Post-translational protein phosphorylation", "Assembly of collagen fibrils and other multimeric structures", "Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)", "Defective CHST14 causes EDS, musculocontractural type", "Defective CHST3 causes SEDCJD", "ECM proteoglycans", and "Platelet degranulation" (Supplemental Table S1). We then created the reaction map according to the signaling pathways (Figure 4).

Potential inhibitors between the young and aging skin

To further know the potential regulator inhibitors, we introduced the L1000FDW tool to predict the potential inhibitors. We selected the top ten molecules according to the DEGs and the inhibitor map: "WZ-3105", "BRD-K33045404", "trametinib", "forskolin", "mycophenolic-acid", "BRD-K60297835", "HG-6-64-01", "BRD-K68548958", "mocetinostat", and "palbociclib" (Figure 5 and Supplemental Table S2).

Discussion

Aging is a progressive loss of organ functions[1]. Recently, skin changes with aging are the major focus in recent medical issues[4]. Thus, knowledge of skin metabolism during aging will deepen our understanding in future studies.

To understand the aging effects on the skin, we analyzed the skins from aged people and young people. By analyzing the DEGs[15, 16], we selected 10 proteins that may be crucial for skin aging according to the PPI networks. Fibrillin-1 has the ability to regulate the fibrotic phenotype through mediating the integrin binding[17]. SPARC can accelerate cutaneous wound closure and mediate the processing of collagen fibrillogenesis in dermal fibroblast[18]. Thrombospondin-1 is known as an anti-inflammatory factor that prevents UVB-induced carcinogenesis[19]. NF-κB is a central factor in inflammation that involves numerous physiological and pathophysiological processes[20–23]. Interestingly, Thrombospondin-1 can regulate inflammatory cytokines through NF-kB signaling[24]. Decorin has the ability to control the collagen matrix

assembly, which plays a role in skin aging[25]. COL1A2 mainly maintains the bone and skin structure[26]. Versican is an important extracellular matrix regulator of immunity and inflammation[27]. Lox is a potential trigger of cardiovascular diseases[28]. SERPING1 gene mutation can drive severe swelling of the skin[29]. FSTL1 showed the molecular functions in arthritis and immune diseases[30]. A study showed that Fibulin-5 is related to pseudoexfoliation[31].

KEGG and GO analyses showed that extracellular matrix organization played critical roles in the progression of skin aging. The extracellular matrices (ECMs) provide physical scaffolds and control many cellular processes including migration, differentiation, and survival[32]. G protein-coupled receptors (GPCRs) contain the largest family of receptors. Activation of GPCRs involves numerous physiological functions and signaling pathways[33–39]. Recently, GPCR was reported to activate the protease and regulate the ECM[40]. Circadian gene clocks and their controlled proteins maintain the tissue homeostasis[41, 42] and involve almost all the biological processes including metabolism, inflammation, apoptosis, and aging[43–48]. Moreover, Charles H Streuli et al. reported that the intrinsic circadian clock can affect the extracellular[49]. Age-related alterations in skin homeostasis can be found at the cellular and tissue levels, which can also be affected by various of factors[50]. Thus, we predicted the extracellular matrix and cell-cell interaction may be important targets during aging.

Briefly, we identified the potential DEGs and pathways for the aging skin. Extracellular matrix organization is the most affected process during aging. Our study provides further insights into the mechanism of aging skin.

Declarations

Author Contributions

Jing Wang: Methodology and Writing. Hanming Gu: Conceptualization, Methodology, Writing- Reviewing and Editing.

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Declarations of interest

There is no conflict of interest to declare.

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Tables

Tables 1-2 are available in the Supplementary Files section.

Figures

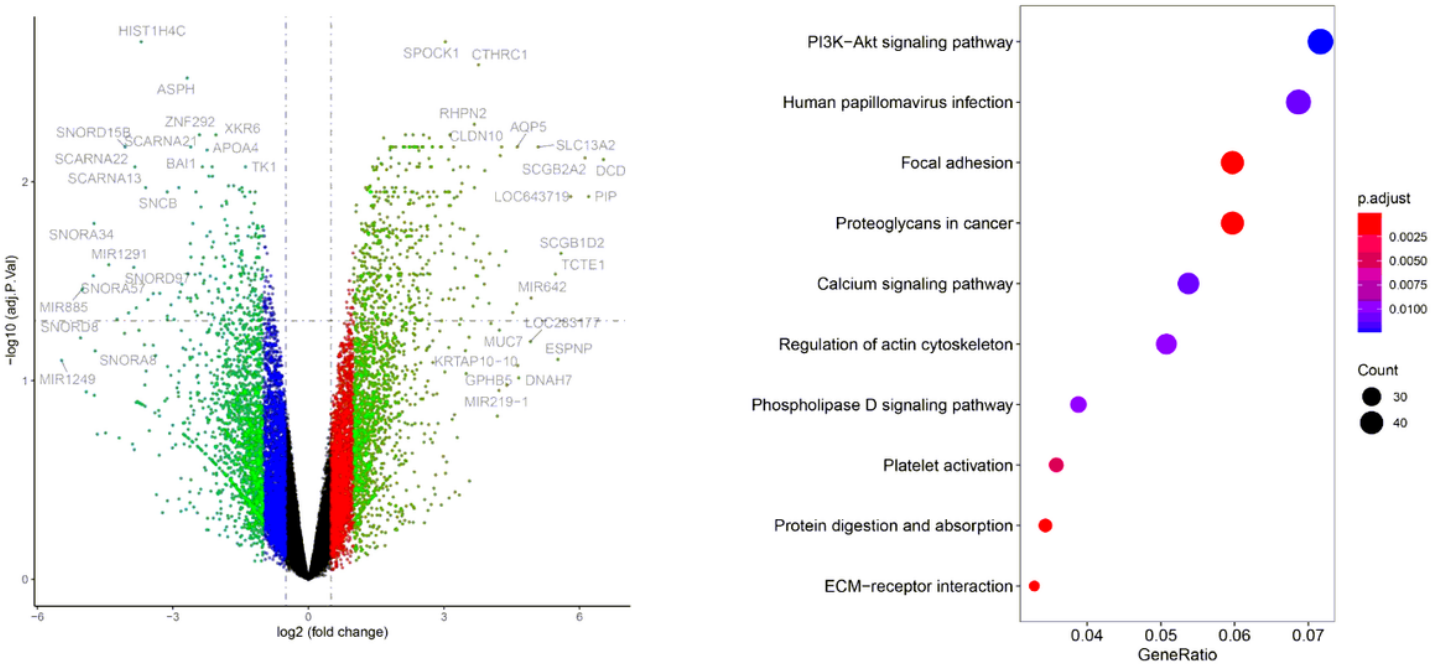

Figure 1

Volcano plots and KEGG analysis Left: Volcano plots depicting gene expression changes in aged skin as compared to control cells. Right: The KEGG pathways enriched by the DEGs.

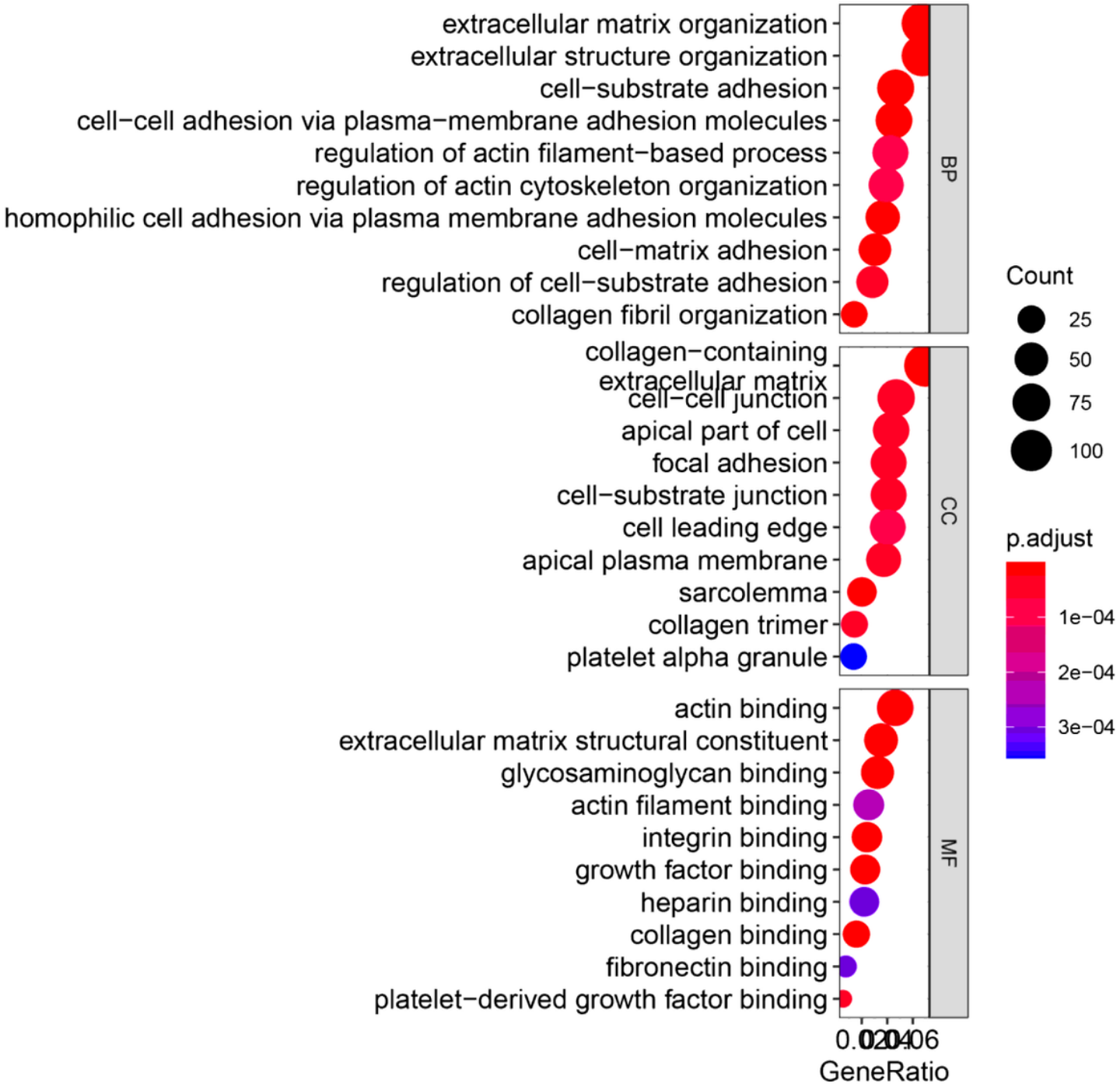

Figure 2

The biological process (BP), cellular component (CC), and molecular function (MF) terms enriched by the DEGs

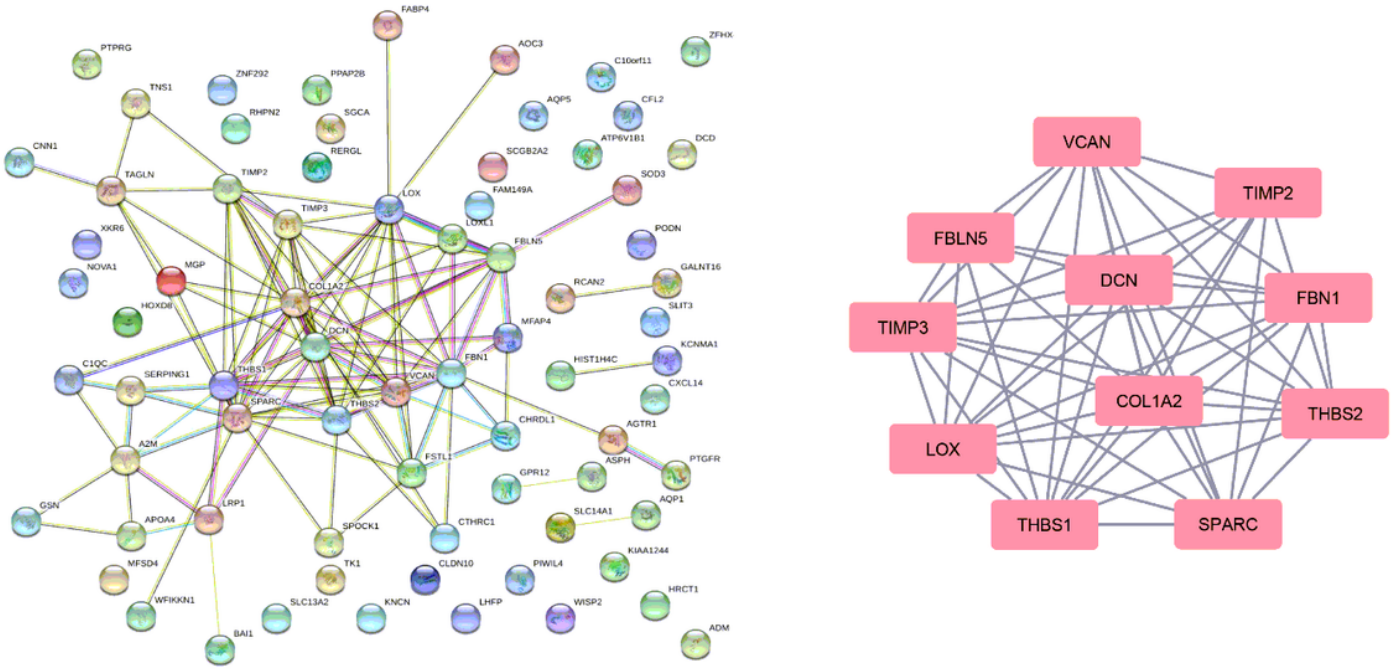

Figure 3

The PPI and top cluster were depicted by String networks

Figure 4

The Reactome pathway analysis Input genes are from the GSE39170 dataset (P <0.001). The yellow color represents the most relevant signaling pathways.

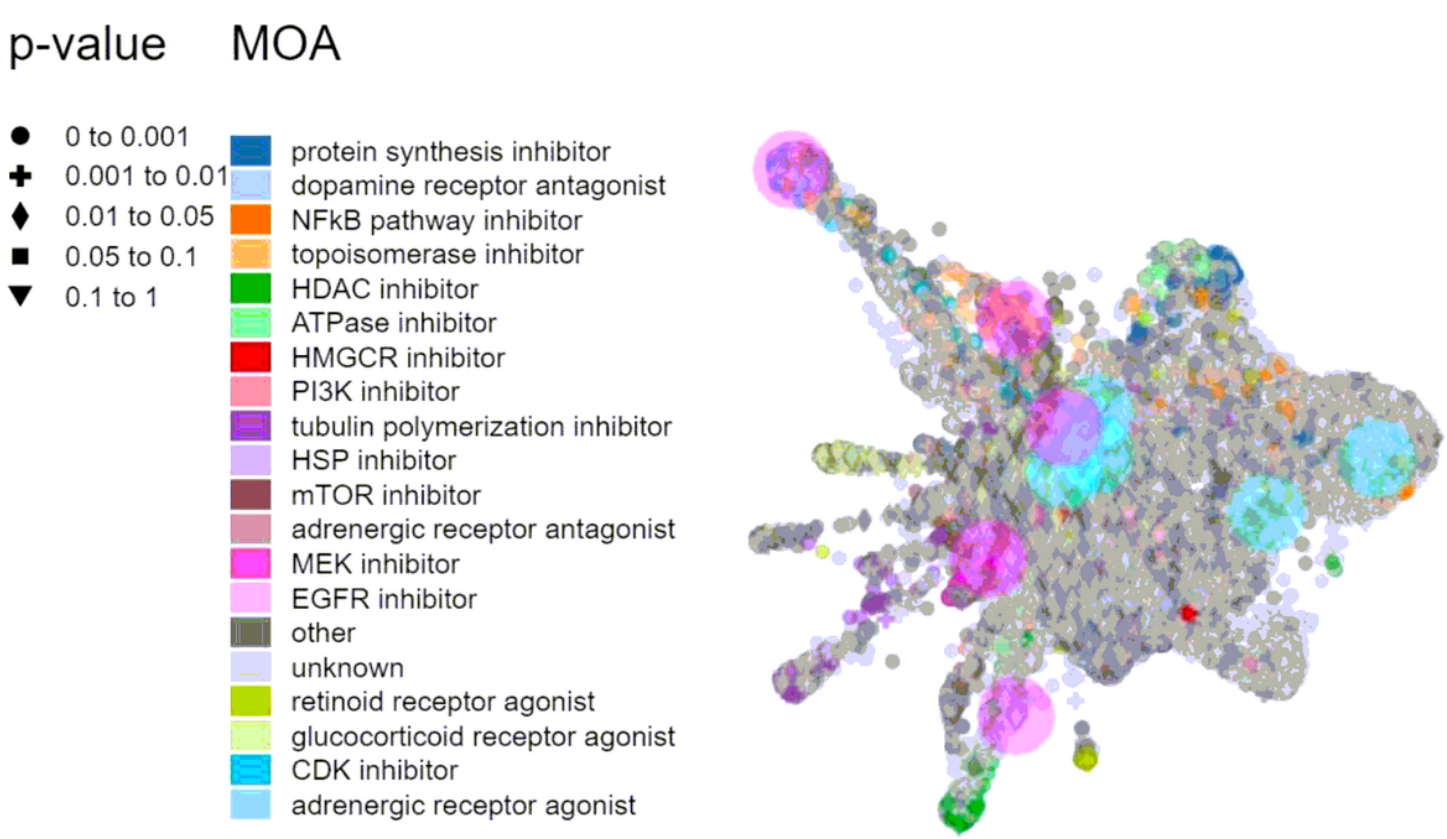

Figure 5

Inhibitors by L1000FDW visualization Input genes are showed by the significantly changed genes obtained from the GSE39170 dataset. Dots are the Mode of Action (MOA) of the respective drug.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTableS1.xlsx
- SupplementalTableS2.xlsx
- Onlinefloatimage6.png
- Onlinefloatimage7.png