Original Article

Identifying the focuses of hereditary gingival fibromatosis with bioinformatics strategies

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Abstract: Objective: The objective of this study was to detect the undiscovered bioinformatics information about hereditary gingival fibromatosis and find focuses from published datasets. Methods: Two published datasets containing gingival tissue expression profiles of HGF and healthy groups were collected from GEO database. GSE4250 was utilized for cardinality analysis, including the differentially expressed gene analysis, enrichment analyses, hierarchical clustering analysis, and protein-protein interaction network. Key genes were obtained from the protein interaction network plot. GSE58482 was utilized for validation. Results: Analysis of the expression profiling by array, there were 785 genes (380 upregulated genes, 405 downregulated genes) expressed differentially between HGF gingival tissue and healthy gingival tissue. KEGG and GO enrichment analyses obtained candidate pathways. Differentially expressed genes were associated with activated pathways like skin barrier pathway and cornified envelope pathway. Repressed pathways included ion homeostasis pathway, receptor ligand activity pathway, and cell population proliferation pathway. Key genes such as F2R, TGM7, and MMP13 were confirmed with differential expression by external validation. Conclusion: By bioinformatics approaches, we found new discoveries including several pathways and key genes. These discoveries deserve attention and research in the future.

Keywords: Hereditary gingival fibromatosis, differentially expressed genes, RNA, bioinformatics

Introduction

Hereditary gingival fibromatosis (HGF) is a genetically heterogeneous disease manifested by slowly progressive, benign, localized, or generalized overgrowth of the gingiva [1]. No effective non-invasive methods are available in the aspect of treatment [2]. The overgrowth gingiva can cover the crown of teeth, resulting in diastemas, teeth displacement or retention of primary dentition, disorders of speech or masticatory, or psychological issues [3].

HGF can present as an isolated condition or co-exist with other genetic syndromes such as Jones syndrome, Ramon syndrome, and Zimmermann-Laban syndrome [3]. In this study, non-syndromic hereditary gingival fibromatosis was chosen to be the major research object.

HGF is considered to be inherited as an autosomal dominant or autosomal recessive trait [1]. Various research report there are related chromosomal regions like 5q13-q22, 2p21-p22, 11p15, and 2p23.3-p22.3 [4-7]. Gene loci such as GINGF2, GINGF3, and GINGF4 are defined as candidate loci of HGF [5-7]. Two genes, REST and SOS1, have been reported with a clear connection to the process HGF [2, 8, 9].

Studies have attempted to clarify the occurrence and development of HGF, but the exact molecular and biochemical mechanisms have not been completely discovered. The purpose of this study is to find the undiscovered bioinformatics information about HGF based on gingival tissue expression profiles.

Materials and methods

Overall analysis procedure

A comprehensive search was performed through Gene Expression Omnibus (GEO) database. The inclusion criteria were as follows: 1. Expression profiling by array; 2. Homo sapiens; 3.
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![Diagram showing the major flow chart of the study.](image)

**Kyoto Encyclopedia of Genes and Genomes analysis**

Based on the differentially expressed gene analysis, Kyoto Encyclopedia of Genes and Genomes enrichment analysis [10] was conducted. The latest KEGG pathway annotations were obtained through KEGG rest API and adopted as the mapping background. Terms with a FDR of < 0.20 and a p-value < 0.05 were collected as the KEGG result.

**The Gene Ontology analysis**

Based on the differentially expressed gene analysis, the Gene Ontology (GO) analysis [11] was conducted through http://metascape.org [12]. Annotations in GO Biological Processes, GO Cellular Components, and GO Molecular Functions were selected as the mapping background. P-values were selected as the mapping background. P-values were calculated according to the accumulative hypergeometric distribution. Terms with an enrichment factor > 1.5 and a p-value < 0.05 were collected as the GO enrichment result.

**Hierarchical clustering of the enrichment results**

Based on the Gene Ontology analysis, hierarchical clustering analysis was performed through http://metascape.org [12]. For the enriched GO terms, the similarities were estimated according to Kappa scores. Sub-trees with a similarity of > 0.3 were considered to be merged.

**Protein-protein interaction network**

Preliminary protein-protein interaction (PPI) networks were constructed with the search tool for the retrieval of interacting genes (STRING version 11.5) [13]. The Cytoscape version 3.9.1 was used for processing and visualizing the final PPI networks. The edges indicated physical protein associations. The minimum required interaction score was the confidence value of >

Tissue specimens were obtained from hereditary gingival fibromatosis patient gingiva and normal gingiva. Before the end of January, 2022, there were two eligible genetic datasets (GSE4250 [6], GSE58482). Both datasets contained 4 samples. As shown in Figure 1, the series matrix file of GSE58482 was utilized for further analysis and the other dataset was used for validation.

**Differentially expressed gene analysis**

Using R software version 4.1.2, all probe IDs in series matrixes were replaced with the gene symbol according to different GPL platforms separately. GSE 4250 was analyzed in this stage. The eBayes function was used to calculate the moderated t-statistics and log-odds of differential expression. Based on the cut-off threshold of p-values < 0.05 and |fold change| > 3, differentially expressed genes between the two groups were analyzed with the linear models’ limma package. The volcano plot and heat map were plotted to visualize the result.
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0.4. Key genes were selected from the PPI networks.

Validation of key genes

GSE58482 was used to validate the key genes. The expression condition was analyzed with R software and the linear models' limma package. The eBayes function was used to calculate the moderated t-statistics and log-odds of differential expression. The overall condition of RNA was plotted by the volcano plot. Key genes were marked out on the volcano plot.

Results

Based on GSE4250, 785 differentially expressed genes, 380 upregulated genes and 405 downregulated genes, were identified with the cut-off threshold of p-values < 0.05 and |fold change| > 3 (Figure 2). The heat map of differentially expressed genes is shown in Figure 3.

In Kyoto Encyclopedia of Genes and Genomes analysis (Figure 4), upregulated differentially expressed genes were enriched in circadian entrainment, dopaminergic synapse, nicotine addiction, neuroactive ligand-receptor interaction, GABAergic synapse, taurine and hypo-taurine metabolism, arachidonic acid metabolism, SNARE interactions in vesicular transport, retrograde endocannabinoid signaling, glutamatergic synapse, glycosphingolipid biosynthesis-globo and isoglobo series, and serotonergic synapse. Downregulated differentially expressed genes were enriched in rheumatoid arthritis, cytokine-cytokine receptor interaction, hypertrophic cardiomyopathy, IL-17 signaling pathway, viral protein interaction with cytokine and cytokine receptor, renin-angiotensin system, neuroactive ligand-receptor interaction, malaria, maturity onset diabetes of the young, adrenergic signaling in cardiomyocytes, autoimmune thyroid disease, and cardiac muscle contraction.

The results of GO are displayed in Figure 5. Applying 0.3 kappa score as the threshold, the
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such as F2R, GYS2, CXCL6, and MMP13, were identified and represented in red (Figure 7).

Identified differentially expressed genes were examined in GSE58482. As shown in the volcano plot (Figure 8), these key genes were marked out. TGM7, SERPINB3, MMP13, and GYS2 met the condition of |fold change| > 3 subsets of upregulated and downregulated representative terms are respectively clustered (Figure 6A, 6B).

In terms of protein-protein interaction network, upregulated differentially expressed genes and downregulated differentially expressed genes were analyzed respectively. Key gene nodes, such as F2R, GYS2, CXCL6, and MMP13, were identified and represented in red (Figure 7).
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Figure 5. A. The Gene Ontology analysis result of upregulated genes. B. The Gene Ontology analysis result of downregulated genes.

Figure 6. A. The hierarchical clustering plot of the Gene Ontology analysis of upregulated genes. B. The hierarchical clustering plot of the Gene Ontology analysis of downregulated genes.
Figure 7. A. The protein-protein interaction network plot of upregulated genes. B. The protein-protein interaction network plot of downregulated genes. Red nodes indicate the key genes.
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and p-values < 0.01. CXCL1 and CA2 met the condition of \(|\text{fold change}| > 2\) and p-values < 0.01. TRPV6, TMEM45A, and F2R met the condition of \(|\text{fold change}| > 1.5\) and p-values < 0.05.

Discussion

Relevant literature have shown that HGF gingiva is linked with nonspecific histological features, like the irregular gingival epithelium, randomly arranged increments of collagen, and elongated rete ridges extending into the underlying connective tissue [14, 15]. The pathological gingival tissue hinder oral cavity hygiene and provide conditions for the reproduction of microorganisms, plaque accumulation, pseudo pocket formation, and gingival inflammation [16].

In the explanation of this pathological process, there have been theories including the epithelial to mesenchymal transition [17] and alterations in the expression of matrix metalloproteinases (MMPs) [3, 18]. The molecular mechanisms of HGF have not been discovered.

Our transcriptome analyses were performed on the foundation of the expression profile of two GEO datasets. According to GSE4250, there were 380 upregulated genes and 405 downregulated genes between the hereditary gingival fibromatosis group and the healthy group. We investigated the output of enrichment analyses and found that the upregulated differentially expressed genes were enriched in transmitter-gated ion channel activity, corneal envelope, glutamate metabolic process, arachidonic acid metabolic process, ATP-dependent activity, G-protein beta-subunit binding, dynein axonemal particle, G protein-coupled peptide receptor activity, membrane lipid metabolic process, regulation of small GTPase mediated signal transduction, establishment of skin barrier, glutamate receptor activity, reciprocal meiotic recombination, sensory perception of mechanical stimulus, dopaminergic synapse, neuroactive ligand-receptor interaction, GABAergic synapse, taurine and hypo-taurine metabolism, SNARE interactions in vesicular transport, retrograde endocannabinoid signaling, and glutamatergic synapse. In terms of the downregulated gene enrichment analysis, GO contained blood circulation, receptor ligand activity, cell population proliferation, muscle system process, integral component of postsynaptic membrane, body morphogenesis, ion homeostasis, endochondral bone growth, metal ion transport, hemoglobin complex, second-messenger-mediated signaling, sarcoplasmic reticulum calcium ion transport, apical plasma membrane, and regulation of body fluid levels. Neither KEGG nor GO have established a specific independent enrichment pathway for HGF. We found irrelevant results like retinal ganglion cell axon guidance pathway, arthritis pathway and hypertrophic cardiomyopathy pathway. We also found related pathways. The enrichment of KEGG and GO were different. Only arachidonic acid metabolism was identified by both analyses. The arachidonic acid metabolism pathway modulates gene expression, ion transport, and anti-inflammatory processes [19], which can verify the fact that HGF is usually developed.
with mild chronic inflammatory. In the pathologic state of HGF, gingival tissues were related with lower levels of ion homeostasis, receptor ligand activity, cell population proliferation, metal ion transport, second-messenger-mediated, and sarcoplasmic reticulum calcium ion transport pathways. Although the development of HGF is generally regarded to be related to genetic factors, the maladjustment of ion homeostasis such as the cell membrane in the Ca²⁺/Na⁺ ion flow and active cationic transport and on passive diffusion can also take a role in the dysfunctional degradation of the overgrowth gingival tissue [20]. The establishment of skin barrier pathway and cornified envelope pathway can corroborate the gingival epithelial change in HGF. Regular metabolic pathways like ATP-dependent activity, membrane lipid metabolic process, transmitter-gated ion channel activity, and SNARE interactions in vesicular transport were also activated in the pathologic state of HGF.

External validation was performed based on GSE58482. According to the volcano plot (Figure 8), downregulated key genes like SERPINB3, TGM7, and CXCL1 were confirmed with less expression. Uregulated key genes such as F2R, TRPV6, and ACSM3 were also confirmed. TGM7, F2R, SERPINB3, MMP13, GYS2, TMEM45A, and TRPV6 met the condition of |fold change| > 2 and p-values < 0.01. At the protein level, MMP13 belongs to Matrix metalloproteinases, a family of zinc-dependent endopeptidases that degrade various proteins in the extracellular matrix [21]. The decrease in the expression of MMPs has been observed in fibroblasts from HGF patients [18]. The downregulated of MMP13 in HGF gingival tissue can explain the excessive accumulation of extracellular matrix proteins, which is considered as the core pathologic manifestation of HGF [14]. Other key genes have not been investigated in the research of HGF. For instance, TGM7 belongs to the transglutaminase superfamily and catalyzes the cross-linking of proteins and the conjugation of polyamines to proteins. F2R, a member of the G-protein-coupled receptor, plays an important role in inflammation and coagulation at the protein level [22]. Other key genes identified with the exact effects in HGF remain to be explored.

There were limitations in this study. There were only two available datasets with small sample sizes. With the popularization of the gene chip technique, we believe more big-sample researches on HGF will be conducted. The result of this paper provided the reference and comparison for future bioinformatics analysis.

Conclusion

Analysis of the expression profiling by array, we found differences between HGF gingival tissue and healthy gingival tissue at the RNA level. Differentially expressed genes were associated with pathways like skin barrier pathway, cornified envelope pathway, and arachidonic acid metabolism pathway. Key genes such as F2R, TGM7, and MMP13 were confirmed with differential expression by external validation. These key genes deserve further attention. More detailed research are required in the future.

Disclosure of conflict of interest

None.

Abbreviations

HGF, Hereditary gingival fibromatosis; GEO, Gene Expression Omnibus; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; STRING, The search tool for the retrieval of interacting genes; MMPs, Matrix metalloproteinases; API, Application program interface.

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