Bioinformatics-Based Study to Investigate Potential Differentially Expressed Genes and miRNAs in Hashimoto's Thyroiditis

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Bioinformatics-Based Study to Investigate Potential Differentially

Expressed Genes and miRNAs in Hashimoto's Thyroiditis

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Abstract

Background: Hashimoto's thyroiditis (HT), also known as chronic lymphocytic thyroiditis, is a common autoimmune disease, which mainly occurs in women. The early manifestation was hyperthyroidism, however, hypothyroidism may occur if HT was not controlled for a long time. Numerous studies have shown that multiple factors, including genetic, environmental, and autoimmune factors, were involved in the pathogenesis of the disease, but the exact mechanisms were not yet clear. The aim of this study was to identify differentially expressed genes (DEGs) by comprehensive analysis and to provide specific insights into HT.

Methods: Two gene expression profiles (GSE6339, GSE138198) about HT were downloaded from the Gene Expression Omnibus (GEO) database. The DEGs were assessed between the HT and normal groups using the GEO2R. The DEGs were then sent to the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The hub genes were discovered using Cytoscape and CytoHubba. Finally, NetworkAnalyst was utilized to create the hub genes' targeted microRNAs (miRNAs).

Results: A total of 62 DEGs were discovered, including 60 up-regulated and 2 down-regulated DEGs. The signaling pathways were mainly engaged in cytokine interaction and cytotoxicity, and the DEGs were mostly enriched in immunological and inflammatory responses. IL2RA, CXCL9, IL10RA, CCL3, CCL4, CCL2, STAT1, CD4, CSF1R, and ITGAX were chosen as hub genes based on the results of the protein-protein interaction (PPI) network and CytoHubba. Five miRNAs, including mir-24-3p, mir-223-3p, mir-155-5p, mir-34a-5p, mir-26b-5p, and mir-6499-3p, were suggested as likely important miRNAs in HT.

Conclusions: These hub genes, pathways and miRNAs contribute to a better understanding of the pathophysiology of HT and offer potential treatment options for HT.

Keywords: Hashimoto's thyroiditis, hub genes, MicroRNAs, Computational Biology

Introduction

Hashimoto's thyroiditis (HT), also known as chronic lymphocytic thyroiditis, is an autoimmune thyroid disease, characterized by extensive lymphocyte infiltration, fibrosis and parenchymal atrophy of the thyroid tissue. Since HT was first described in 1912 by Dr. Hakaru Hashimoto [1], its prevalence has an increasing trend. HT was considered as the most common autoimmune disease, and the most frequent cause of hypothyroidism [2], diagnosed predominantly in women, the estimated number of new cases of HT was estimated to be around 3.5 per 1000 in women and 0.8 per 1000 in males [3]. The major risk factors of HT were radiation, infections, stress, drugs, excessive iodine, climate, age and gender [4]. Clinical characteristics, including the detection of

TPOAb and/or TGAb, and typical thyroid ultrasonography abnormalities were used to diagnose HT[5].

Hashimoto's thyroiditis was thought to be caused by a combination of factors, including genetic predisposition, environmental influences, and immunological problems[6]. Recent studies have shown that an imbalance in CD4⁺ T lymphocyte differentiation potentially contributed to HT onset[7]. HT has been found to be linked to several gene loci, including immunoregulatory genes (CD40, FoxP3, CD25, NLRC4, CTLA-4) and thyroid-specific genes (TSH receptors and thyroglobulin)[8–10]. According to one research, ten hub genes (LCP2, PTPRC, HLA-DRA, CD3D, HLA-DPB1, HLA-DPA1, CD247, ITK, CD53, and ITGB2) and four critical pathways (IL6/JAK/STAT3, IFN- α , IFN- γ , and inflammatory pathways) have been found to be highly linked to HT[11].

There are no recognized techniques for treating HT since the causes underlying autoimmune disorders are complicated and varied. As a result, physicians need search for effective treatments to cure HT and to prevent HT development before carcinogenic consequences and other complications occur. Biomarkers might be the key to customize therapy in HT, so that patients would be treated differently according to their biological fingerprints. Clinical bioinformatics is considered to be one of the most promising and important technologies to overcome the clinical problems of early diagnosis, effective treatment and prognosis of cancer patients[12]. Bioinformatics research has also revealed a growing number of new biomarkers in several non-neoplastic diseases[13, 14].

We analyzed two original HT datasets which were downloaded from the Gene Expression Omnibus (GEO) datasets for this aim. For further investigation, we identified differential expression genes (DEGs) for functional and pathway enrichment analysis. And DEGs prediction was linked to protein-protein interaction (PPI) analysis, as well as microRNAs (miRNAs). Finally, a gene-miRNA regulatory network was constructed to anticipate possible miRNAs linked with DEGs. A flow chart of the study design is shown in Figure 1.

Figure 1. Flow chart of this study

Results

Identification of DEGs in HT

According to the criterion of adjusted P-value < 0.05 and $|\log FC| > 1$, we identified 681 DEGs from GSE6339 contains 505 up-regulated genes and 176 down-regulated genes compared with normal controls. From GSE138198, we obtained 1771 DEGs, including 1091 up-regulated genes and 680 down-regulated genes compared with normal samples. The cluster of DEGs was shown using a volcano graphic (Figure 2). These genes were further screened by mapping Venn diagrams. As shown in Figure 3, 62 DEGs were determined to be significantly differently expressed between the two datasets, with 60 up-regulated genes and 2 down-regulated genes.

Figure 2. Volcano plot representing differential expression genes (DEGs) between HT and normal groups. (A) DEGs in GSE6339; (B) DEGs in GSE138198.

Figure 3. Venn diagrams showing the overlaps of numbers of DEGs. GSE6339-UP, GSE6339-DOWN respectively represent up-regulated and down-regulated genes in GSE6339. GSE138198-UP, GSE138198-DOWN respectively represent up-regulated and down-regulated genes in GSE138198.

Functional classification and pathway enrichment analysis of DEGs

We performed GO and KEGG analysis on the 62 DEGs. The top 10 of each GO term and the top 20 KEGG pathways were listed. For GO-BP terms, DEGs were mainly enriched in response to interferon-gamma, cellular response to interferon-gamma, positive regulation of leukocyte activation, positive regulation of cell activation, leukocyte migration, lymphocyte differentiation, neutrophil migration, granulocyte migration, myeloid leukocyte differentiation, myeloid leukocyte migration (Figure 4A). According to the GO-CC terms analysis, DEGs were mostly enriched in integral component of luminal side of endoplasmic reticulum membrane, luminal side of endoplasmic reticulum membrane, clathrin-coated vesicle membrane, MHC class II protein complex, external side of plasma membrane, tertiary granule, MHC protein complex, coated vesicle membrane, clathrin-coated vesicle, coated vesicle (Figure 4B). In GO-MF analysis, DEGs were found to be substantially enriched in chemokine receptor binding, CCR chemokine receptor binding, chemokine activity, peptide antigen binding, cytokine binding, cytokine receptor activity, G protein-coupled receptor binding, cytokine receptor binding, cytokine activity, protein tyrosine kinase binding (Figure 4C). As shown in Figure 5, the KEGG pathway analysis indicated that the DEGs were predominantly enriched in Cytokine-cytokine receptor interaction, Hematopoietic cell lineage, Th1 and Th2 cell differentiation, Epstein-Barr virus infection, Chemokine signaling pathway, Viral protein interaction with cytokine and cytokine receptor, Th17 cell differentiation, Human T-cell leukemia virus 1 infection, Tuberculosis, Human immunodeficiency virus 1 infection, Cell adhesion molecules, Antigen processing and presentation, Influenza A, Measles, Toxoplasmosis, Chagas disease, Staphylococcus aureus infection, Rheumatoid arthritis, Inflammatory bowel disease, Asthma (Figure 5).

PPI network analysis and hub genes identification

A total of 62 DEGs (60 up-regulated genes and 2 down-regulated genes) were filtered into a PPI network using the STRING online database and visualized by Cytoscape software (Figure 6). Based on the node degree score provided by Cytoscape's cytohubba plugin, the top 10 genes, including IL2RA, CXCL9, IL10RA, CCL3, CCL4, CCL2, STAT1, CD4, CSF1R, and ITGAX, were chosen as possible hub genes (Figure 7).

Integrated miRNA-gene regulatory networks

NetworkAnalyst datasets were used to predict substantially distinct miRNAs and gene regulatory networks, which were then displayed using Cytoscape. The top 10 DEGs and their matching regulatory miRNAs were shown in Figure 8. ITGAX and IL2RA, for example, might be expected as miRNA targets (hsa-mir-6499-3p). Three target genes (CCL2, CSF1R, STAT1) might be bound by the miRNAs (hsa-mir-155-5p). These findings need further verification.

Figure 4. Gene Ontology (GO) functional analysis of DEGs. GO functional analysis showing enrichment of DEGs in (A) biological process, (B) molecular function, and (C) cellular component.

Figure 5. KEGG pathway enrichment analysis of DEGs

Figure 6. Protein-protein interaction (PPI) network of DEGs

Figure 7. Protein-protein interaction network for the top 9 hub genes.

Figure 8. Integrated DEGs-miRNAs networks for the top 10 hub genes.

Discussion

The incidence of HT is increasing, researches about the etiology and diagnostic biomarkers are required for the early diagnosis and treatment. As an autoimmune disease, the specific thyroid antibodies of HT were produced by malfunction of B and T cells[7, 15]. The pathogenic mechanism of HT is multifactorial, with environmental factors, genetic factors and immune disorders all combining to create various clinical symptoms[6]. This study may give some new insights for further HT research.

In this project, we downloaded two datasets about HT from the GEO datasets for analysis. A total of 62 DEGs were identified, including 60 up-regulated genes and 2 down-regulated genes. The results of GO functional classification indicated that the DEGs were most concentrated in positive regulation of leukocyte activation, chemokine receptor binding, CCR chemokine receptor binding, chemokine activity involved in immune response. The pathways enrichment analysis showed that DEGs mainly enriched in inflammatory response and immune receptor-related signaling pathways. We identified the top 10 hub genes (IL2RA, CXCL9, IL10RA, CCL2, CCL3, CCL4, STAT1, CD4, CSF1R, ITGAX) that may influence the onset or development of HT using PPI network analysis.

Interleukin-2 receptor alpha (IL2RA) is one of three subunits that make up the high affinity interleukin 2 receptor (IL-2R)[16]. The IL2RA gene, which is found on chromosome 10p15, encodes CD25 and influences the generation and function of regulatory T cells by acting as a differentiation factor and inhibiting autoreactive T cells in the periphery via CD25[17]. Beata et al[18] found the rs7093069-IL2RA genotype may increase the risk of autoimmune thyroid diseases.

Chemokine (C-X-C motif) ligand 9 (CXCL9) is a tiny cytokine that belongs to the CXC chemokine family and also known as monokine induced by gamma interferon (MIG). CXCL9 is involved in initiating chemotaxis, increasing leukocyte differentiation and proliferation, and producing tissue extravasation[19]. In various diseases, its expression correlates with the tissue infiltration of T cells[20]. According to certain studies, CXCL9 causes Th1 polarization via

CXCR3, and subsequently stimulates immune cells via Th1 polarization and activation[21].

Interleukin 10 receptor, alpha subunit (IL10RA) is a subunit of the interleukin-10 receptor. IL10 was a crucial anti-inflammatory cytokine that inhibits the pro-inflammatory responses of innate and adaptive immune cells[22]. Some reports indicated that IL10RA was related to early onset-inflammatory bowel disease[23], anaplastic large cell lymphoma[24], colorectal cancer[25].

Chemokine (C-C motif) ligand 2 (CCL2) was also known as monocyte chemotactic protein 1 (MCP1) and small inducible cytokine A2. Chemokine (C-C motif) ligand 3 (CCL3), also known as macrophage inflammatory protein 1-alpha (MIP-1- α), was involved in the acute inflammatory state in the recruitment and activation of polymorphonuclear leukocytes through binding to the receptors CCR1, CCR4 and CCR5[26]. Chemokine (C-C motif) ligands 4 (CCL4), also known as Macrophage inflammatory protein-1 β (MIP-1- β), was a chemoattractant for natural killer cells, monocytes and a variety of other immune cells[27]. CCL2, CCL3, CCL4 all belong to the CC chemokine family and their genes in humans are localized on chromosome 17[28]. CCL2 has been reported to be related to osteoarthritis, HIV-1 infection, cancer metastasis and HT[29–32]. Some researches found the levels of CCL3 and CCL4 transcripts were significantly improved in autoimmune thyroid glands when compared to non-autoimmune thyroid glands[33].

Signal transducer and activator of transcription 1 (STAT1) is a transcription factor which is encoded by the STAT1 gene in humans. STAT1 could be activated by several ligands such as INF- α INF- γ , EGF, PDGF, IL-6 or IL-27[34]. Due to its activation of the transcription factor T-bet, STAT1 was recognized to be crucial for the production of Th1 cells downstream of INF- γ [35]. STAT1 was involved in the signaling of IFN and a variety of interleukins, making it an important part of the normal immune response and also has a role in the shape and function of the thyroid[36].

Cluster of differentiation (CD4) is a glycoprotein that functions as a co-receptor for the T-cell receptor, which is encoded by the CD4 gene[37]. Th17 cells and Th22 cells were CD4+ T-cell lineages, and a recent study found a significant connection between Th22 and Th17 levels in HT tissues, suggesting that Th22 and Th17 cells were linked to the pathogenesis of HT[38].

Colony stimulating factor 1 receptor (CSF1R), also known as macrophage colony-stimulating factor receptor (M-CSFR), and CD115 (Cluster of Differentiation 115), is a cell-surface protein. The protein belongs to the CSF1/PDGF receptor family of tyrosine-protein kinases and is a transmembrane tyrosine kinase[39]. CSF1R was an oncology therapeutic target that suppresses paracrine mechanisms in the tumor microenvironment or recanalizes tumor-associated macrophages (TAMs)[40]. According to one research, IL-34 has a role in thyrocyte apoptotic resistance in HT caused by CSF-1R and might be used to assess thyrocyte damage[41]. For this reason, CSF1R may be a potentially new treatment target for HT.

Integrin alpha X (ITGAX), also named as CD11b, encodes the integrin α -X chain protein, which forms by association with β -chain[42]. This integrin has similar activities with α M β 2, such as the mediation of phagocytosis and of leukocyte adhesion and migration by binding to fibrinogen and ICAM ligands[43].

MiRNAs play a role in controlling gene expression by degrading and weakening the translation of their target genes. There were several studies that have looked into the relationship between miRNAs and hub genes. For instance, Peng and colleagues found that down-regulated miR-125a-3p expression in thyroid tissues from HT patients, which might be involved in the

pathogenesis of HT[44]. Zhao and associates indicated that 6 miRNAs (miR-205, miR-20a-3p, miR-375, miR-296, miR-451, miR-500a) were significantly up-regulated in peripheral plasma of HT patients compared with normal controls[45]. MiR-21-5p, miR-146b-3p, miR-5571-3p and miR-6503-3p were also thought to be related to HT[46]. Several miRNAs, including mir-24-3p, mir-223-3p, mir-155-5p, mir-34a-5p, mir-26b-5p, and mir-6499-3p, may play a key role in HT, according to our findings. However, the roles of mir-24-3p, mir-155-5p, mir-34a-5p, mir-26b-5p, and mir-6499-3p in HT need to further research. Furthermore, there is currently a scarcity of study on the genes and miRNAs involved in HT.

The gene-miRNA regulatory network plays an important role in the pathophysiology of HT. This discovery will help us better understand the mechanism of HT and provide effective and novel treatment strategies for HT. Nonetheless, the current study has several drawbacks. First, only two datasets containing high-throughput sequencing data for HT was selected for analysing. At present, most databases, such as TCGA and GEO, mainly provide high-throughput microarray data for cancer-related research, few databases provide data on non-tumor illnesses (such as coronary artery disease and diabetes). As a result, additional study on HT using microarrays and high-throughput sequencing is required. Second, there is a scarcity of study into the exact molecular processes by which hub genes and miRNAs control HT. Further molecular biology experiments in the follow-up work to verify the function of hub genes at the cell or specimen level were needed.

Conclusions

The expressions of IL2RA, CXCL9, IL10RA, CCL2, CCL3, CCL4, STAT1, CD4, CSF1R, and ITGAX in patients with HT were shown to have a significant impact on the pathophysiological process of HT when compared to the healthy control group. Mir-24-3p, mir-223-3p, mir-155-5p, mir-34a-5p, mir-26b-5p, and mir-6499-3p were all predicted as possible target miRNAs. The discovery of these genes and miRNAs might help with the development of early detection methods, prognostic indicators, and therapeutic targets for HT. More data and studies are needed to confirm our conclusions about the origins and evolution of HT.

Materials and Methods

Gene expression data selection

Two microarray datasets (GSE6339, GSE138198) of HT and control samples were collected from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>). Among them, GSE6339 was based on GPL4512 TAGC_H.Sapiens_9K_20041223, GSE138198 was based on GPL6244[hugene-1_0-st] Affymetrix human gene 1.0 ST array [transcript (gene version)]. The GSE6339 dataset contains 5 HT samples and 15 normal thyroid samples. GSE138198 includes 13 HT samples and 3 control thyroid tissue samples.

Identification of differentially expressed genes(DEGs)

GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r>) was applied to perform DEGs analysis between HT and normal samples. Genes with adjusted P-value <0.05 and |logFC| >1 were deemed as DEGs. The volcano plots and Venn diagrams of DEGs were generated by R packages.

Functional and pathway enrichment analysis of DEGs

The Gene Ontology (GO) classification, which contains biological processes (BP), cellular component (CC), and molecular functions (MF), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed on the DEGs using the R package. P-values < 0.05 were defined as statistically significant.

Protein–protein interaction (PPI) construction and identification of hub genes

We used the online tool Search Tool for the Retrieval of Interacting Genes (<https://string-db.org/>) database to evaluate PPI information. The minimum required interaction score was medium confidence (0.400). Cytoscape software was used to create the PPI network visualization. Using CytoHubba in Cytoscape to screen hub genes in the PPI network, and the top 10 hub genes were chosen for further study.

Gene-miRNA regulatory network

Using NetworkAnalyst 3.0 (<https://www.networkanalyst.ca/>), a visual online tool, to find miRNA-gene interactions in Gene Regulatory Networks. Lastly, these hub genes and miRNA regulatory network visualization was constructed by Cytoscape.

Abbreviations

HT: Hashimoto's thyroiditis

DEGs: Differentially expressed genes

GEO: the Gene Expression Omnibus (GEO) database

GO enrichment analysis: the Gene Ontology (GO) enrichment analysis

KEGG pathway enrichment analysis: the Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis

PPI: Protein–protein interaction

BP: biological processes

CC: cellular component

MF: molecular functions

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Declarations

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Contributions

L.G.Q. and Q.X.G. came up with the research idea while L.G.Q. and D.J.W conducted the data analyses. In addition, H.Q. and F.X.H contributed significantly to processing the data. L.G.Q. , J.F.H. and G.M. wrote the manuscript. All the authors read and approved the final manuscript.

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Ethics approval and consent to participate

The research is a bioinformatics research.

Consent for publication

Not applicable.

Availability of data and material

The raw data contained expression matrix and GPL information downloaded from the GEO database.

The relevant codes are available upon request

Competing interests

No