Exploring Immune Infiltration and Biomarkers of Ollier Chondrosarcoma

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Research

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Abstract

Background

Ollier disease (OD) is a kind of rare and non-hereditary orthopaedics disease. The malignancy transformation towards chondrosarcoma can cause catastrophic consequences. Our study aimed to reveal the potential molecule mechanism and hub genes involving in Ollier chondrosarcomas. The raw data GSE30835 was acquired from Gene Expression Omnibus (GEO). Differentially expressed genes (DEGs) between Ollier chondrosarcoma and healthy groups were identified. After Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs, protein-protein interaction (PPI) network construction, hub genes and significant modules were selected. CIBERSORT analysis was also carried out.

Results

Together, 226 DEGs were identified, which contained 79 downregulated and 147 up-regulated. Functional and pathway enrichment analysis indicated that DEGs were mainly enriched in extracellular matrix (ECM) structural constituent, MHC class II antigen-related mechanism and phagosome pathway. Two significant modules were related to chondrocyte development, MHC class II antigen processing and presentation. COL3A1, VCAN, COL11A1, THBS1, ITGB1, CCL2, CCND1 were viewed as hub genes. CIBERSORT analysis shows that naive B cells and M0 macrophages are of statistical significance.

Conclusion

Our study suggests that COL3A1, COL11A1, VCAN, ITGB1, THBS1, CCL2, CCND1 may be viewed as promising candidate biomarkers of Ollier chondrosarcoma. We also advanced that MHC class II antigen-related immune mechanism should be paid attention in the further experiment. Naive B cells and M0 macrophages might be related to immune mechanism.

Background

OD affects only one in 100000 persons [1], which is also called dyschondroplasia, multiple cartilaginous enchondromatosis, enchondromatosis Spranger type I [2]. Chondromas are benign asymptomatic tumours originating from hyaline cartilage. If the tumours arise from the medullary canal, they are called enchondromas [2]. Chondrosarcoma characterized by hyaline cartilaginous neoplastic tissue is the third most common bone tumour [3]. The past survey showed that the rate of malignancy transformation of enchondromas to chondrosarcomas was 25.4% [4]. Except for chondrosarcomas, some other kinds of cancers can also be found in OD like gliomas, juvenile granulosa cell tumors, and acute myeloid leukemia [5]. However, studies about these cancers are few. It is noteworthy that the prognosis of low-grade chondrosarcoma is good, for the tumours rare metastasis [6]. The early diagnosis is difficult but essential, because radical surgery is the only way when it comes to the late-stage [7].

OD might result from signal pathways that control the proliferation and differentiation of chondrocytes [8]. Unbalance in the Indian hedgehog signalling pathway, which has been proved to have some influence on the development of this disease [9]. A cytogenetic finding identified an intercalary deletion, del(1)(p11p31.2), as the only chromosome abnormality in low-grade Ollier chondrosarcoma [10]. The molecule mechanism of transformation toward chondrosarcomas is still unknown, and the related studies are few.

Bioinformatic methods are widely used in the analysis of complex diseases. In this study, we used mRNA expression data of GSE30835 containing Ollier chondrosarcoma from GEO dataset to explore the potential pathogenesis mechanism and key genes in malignancy transformation.

Results

Screening of DEGs

We downloaded GSE30835 from GEO dataset. According to the criteria, we had decided ($P < 0.05$, $|\log_2 \text{fold change}| \geq 1$), 226 DEGs could be selected from GSE30835 datasets. Of all DEGs, 79 genes were downregulated, and 147 genes were upregulated. Subsequently, we made a volcano plot to exhibit DEGs, the top 5 upregulated and downregulated DEGs were also marked in Fig. 1a. Heatmap containing top50 upregulated and downregulated genes was shown in Fig.1b.

Functional Enrichment and Pathway Analysis of DEGs

GO enrichment and KEGG pathway analysis were performed by using clusterProfiler R package. KEGG pathway analysis showed that DEGs mainly enriched for the following pathway terms: phagosome, ECM-receptor interaction, staphylococcus aureus infection (Fig. 2). The top2 results of GO enrichment analysis of up-regulated DEGs are listed. For GO BP analysis, the DEGs were mainly enriched in antigen processing and presentation of peptide or polysaccharide antigen via MHC class II, extracellular structure organization (Fig. 3a). For GO CC analysis, the DEGs were mainly enriched in endoplasmic reticulum lumen and collagen-containing extracellular matrix (Fig. 3b). For GO MF analysis, the DEGs were mainly enriched in extracellular matrix structural constituent and MHC class II protein complex binding are related to up-regulated DEGs (Fig. 3c). According to our criteria, GO enrichment analysis about down-regulated DEGs can only get distinct results in BP (Fig. 3d). The main results of BP were GO terms of detoxification and negative regulation of growth.

Construction of PPI and Hub genes

A network made up of 205 nodes and 353 edges visualized the interaction of DEGs (Fig. 4a). Total 7 genes overlapped in Fig. 4d, that are, COL3A1 (collagen type III alpha 1 chain), VCAN (Versican), COL11A1 (Collagen XI, alpha-1), ITGB1 (Integrin, beta-1), CCL2 (Chemokine (C-C motif) ligand2), CCND1 (Cyclin D1), THBS1 (Thrombospondin1) were seen as hub genes.

Modules selection and analysis

Two significant modules were obtained using MCODE, module1 (Fig. 4b) and module2 (Fig. 4c). In module1, three hub genes which were COL3A1, VCAN, COL11A1 were included. Module2 contained two hub genes, CCND1 and CCL2. Based on the Reactome Pathway Database (<http://reactome.ncpsb.org>), pathway analysis results of two modules were separately shown in Table 1 and Table 2.

Table 1
Reactome pathway analysis of module1.

Pathway	Description	Count in gene set	False Discovery Rate	pVlaue
HSA-8957275	Post-translation protein phosphorylation	14 of 109	1.33×10^{-25}	1.11×10^{-16}
HSA-1650814	Collagen biosynthesis and modifying enzymes	16 of 76	1.33×10^{-25}	1.11×10^{-16}
HSA-1474244	ECM organization	19 of 329	1.33×10^{-25}	1.11×10^{-16}

Table 2
Reactome pathway analysis of module2.

Pathway	Description	Count in gene set	False Discovery Rate	pValue
HSA-2132295	MHC class II antigen presentation	10 of 148	5.22×10^{-13}	5.55×10^{-15}
HSA-168256	Immune System	20 of 2641	2.70×10^{-12}	5.75×10^{-14}
HSA-877300	Interferon gamma signalling	9 of 250	1.40×10^{-9}	4.52×10^{-11}
HSA-1280215	Cytokine-signaling in immune system	13 of 1055	6.73×10^{-9}	2.92×10^{-10}

Immune infiltration analysis

According to the results of CIBERSORT analysis in Fig. 5, we found that the proportion of B cells naive in Ollier chondrosarcoma was higher than healthy groups and was of statistical significance. While, M0

macrophages in the microenvironment of Ollier chondrosarcoma are enriched. These cells can all play important roles in the immune system.

Discussion

Ollier disease mainly occurs in the first decade of life [4]. The most common malignancy of OD is chondrosarcoma. In the present study, we analyzed OD related microarray datasets from the GEO database to screen for DEGs of Ollier chondrosarcoma and identified candidate hub genes. The relationship between immune and Ollier chondrosarcoma was firstly revealed.

KEGG pathway analysis showed that phagosome and ECM-receptor interaction pathways were closely related to DEGs. Phagocytosis is the main process of phagosome pathway, and can be carried out by the cell of relatively large particles (> 0.5 μ m) into vacuoles. [11]. Normal means of phagosome maturation could promote the immune response [12]. In our study, phagosome pathway mainly included 11 DEGs. Among them, HLA-DMB, HLA-DMA and HLA-DRA are all related to MHC Class II antigen. Last studies have shown that ECM-receptor interaction pathway is also up-regulated in prostate cancer tissue [13].

GO enrichment analysis revealed that significant ontology categories of upregulated DEGs included ECM constituent, glycosaminoglycan binding, and antigen processing and presentation of peptide or polysaccharide antigen via MHC class II. Some studies have demonstrated the composition of the micro-environment play a considerable role in the whole procession of tumors [14]. The peritumoral stroma exists in an extensive ECM composed of a lot of molecules, including glycoproteins, and so on [15]. Hyaluronan(HA) influences whole biological activities in the ECM [16]. A previous study found that HA increased significantly in chondrosarcoma patients [17]. The necrotic zone in vivo is a centre where HLA-DR monocytes are gathered and start to transform into chondrosarcoma [18].

Immune filtration analysis helps us understand the proportion of immune microenvironment. In our study, M0 macrophages are of statistical significance. Richert [19] reported that the M0 cells were characterized by high plasticity and they were both tumor-associated macrophages participating the metastatic spreading. B cells naïve has been proved to perform a role in autoimmune disease such as multiple sclerosis[20]. Recent study revealed that C-type lectin CLEC16A expressed in the surface of naïve B cells participated in MHC Class II pathway[21].

7 hub genes were selected from PPI network. Among them, three hub genes COL3A1, COL11A1 and VCAN were in module1. Module1 was mainly enriched in GO terms of chondrocyte development, post-translation protein phosphorylation and ECM organization pathway. Changes in amount and composition of ECM are considered as a biomarker of tumour development [22].

ITGB1 is regarded as the most significant receptor of COL2A1 [23]. It can mediate COL2A1 related effect on chondrocyte hypertrophy [24]. In oral squamous cell carcinoma (OSCC), THBS1 is a tumor specificity ECM protein induced by TGFB1 and improves the migration ability of cancer cells [25]. It does not directly protect chondrocytes but could reduce inflammation [26].

CCND1 and CCL2 belong to module2 in PPI network. Module2 was mainly enriched in GO terms of antigen processing and presentation, and MHC class II antigen presentation pathway. Romeo S [27] reported that CCND1 was lower expression in high-grade central chondrosarcoma, compared with chondromyxoid fibroma. This phenomenon reflected impairment of cell cycle progression and of cell-cell adhesions in malignant tumors. PTHR1 can directly control the activation of the cyclinD1 promoter [28]. The expression of PTHR1 was higher with increasing histological grade in chondrosarcoma [27]. Chemokine ligand2 (CCL2) belongs to the CC chemokine family. CCL2 is related to the migration of chondrosarcoma. A recent study indicated that CCL2 accelerated the migration of chondrosarcoma cells through the CCR2 receptor and NF- κ B signal transduction pathway [29]. These results square with the functional enrichment analysis results of DEGs and can reflect the primary function of DEGs.

By putting the results together, we found that phagosome pathway was a key link in the presentation of antigens MHC Class II, and linked innate and adaptive immunity [30]. The two terms are both related to upregulated DEGs. Chondrosarcomas and central nervous system tumors are seen as tumors with the highest frequency IDH mutations [5]. A previous study revealed that the maturation of type IV collagen which could result in fragile basement membranes was inhibited in IDH1 R132H knock-in mice [31]. IDH1/2 mutation has also been reported in conventional (central and periosteal) and dedifferentiated chondrosarcoma [31]. In our analysis, IDH1 and IDH2 are both slightly up-regulated. COL4A1 encoding type IV collagen was important in our study, and it played the same role as IDH in regulating basement membrane.

A study based on cDNA, comparing OD with solitary chondrosarcoma samples (n = 7, three with OD). There are no statistical difference in biology between them in this study. This study is limited to the samples available are few because OD is sporadic. While, this study found that JunB protein was significantly lower in enchondromas compared with chondrosarcoma. JunB may be identified as a good diagnostic marker for malignancy [32]. Our study can also support this conclusion, JunB is also a DEG in our study.

Conclusions

In conclusion, our study used GSE30835 dataset and showed that genes such as COL3A1, COL11A1, VCAN, ITGB1, THBS1, CCL2 and CCND1 might be importantly associated with the pathogenesis of Ollier chondrosarcoma and can be potentially seen as biomarkers. GO terms of chondrocyte development, MHC class II antigen presentation pathways and ECM-receptor interaction pathways can be potential mechanism of malignancy transformation. This study can help understand the process of Ollier chondrosarcoma and firstly put forward that immune mechanism and immune microenvironment should be paid close attention in the further study. However, further experimental studies are needed to confirm these results.

Materials And Methods

Microarray Data and Data Processing

The gene expression profiles GSE30835 contributed by was acquired from GEO database (<http://www.ncbi.nlm.nih.gov/geo/>), containing 10 Ollier chondrosarcomas samples and 6 normal (growth plate and cartilage) samples, 4 solitary chondrosarcoma samples, 6 enchondromas of OD, 1 solitary enchondroma samples. We used RStudio software based on R language to select samples we required (10 Ollier chondrosarcoma disease samples and 6 normal samples). The gene chip platform of this dataset is GPL6884 Illumina HumanWG-6 v3.0 expression.

Screening of DEGs

Classical Bays t-test in limma R package was applied [10]. In such study, $|\log_2 \text{fold change (FC)}| \geq 1$ and $P < 0.05$ were regarded as the thresholds for identifying significant DEGs. Heatmap and volcano plots were utilized to visualize the expression levels of significant DEGs.

GO Enrichment and KEGG Pathway Analysis of DEGs

G:profile website (<http://biit.cs.ut.ee/gprofiler/convert>) was utilized to match DEGs with official gene symbol before starting to analyze. KEGG pathway analysis of DEGs was performed with the help of the clusterProfiler Rpackage [33]. Gene Ontology (GO) enrichment analysis can clarify the biological meaning in a large number of genes and classification of gene related functions by providing quantitative and statistical output documents. GO terms were made up of three functional groups: biological processes (BP), cellular component (CC), and molecular function (MF) [34]. Furthermore, the enriched functions of DEGs were selected by the two analysis methods, and $P < 0.01$ was considered statistically significant.

PPI Network Construction

The Search Tool for the Retrieval of Interacting Genes (STRING) database was used to establish the potential interaction of DEGs. PPI pairs with a paired core > 0.4 were extracted, and we hid those disconnected nodes in the PPI network. After that, the network file was imported into Cytoscape software (version 3.6.1).

Selection of Hub Genes and Modules Analysis

DEGs in PPI network were evaluated by 5 topological algorithms (Degree, Closeness centrality, Maximum Neighborhood Component, Radiality centrality, Betweenness centrality) in the plug-in cytoHubba (version 0.1) [35]. Then, the plug-in Molecular Complex Detection (MCODE) was applied to cluster the PPI network [36]. Several important modules were selected. Based on the Reactome Pathway Database (<http://reactome.ncpsb.org>), Reactome pathway analysis of the most significant modules was performed [37].

Immune infiltration analysis via CIBERSORT

In order to confirm the immune related cells in Ollier chondrosarcoma, CIBERSORT method was used to analyze the situation of immune infiltration between Ollier chondrosarcoma and normal tissues[38]. Then I used the “ggplot2” and “ggpubr” R packages to show the infiltration level of immune cells as well as the difference between Ollier chondrosarcoma and healthy groups.

Abbreviations

Ollier disease: OD

Differentially expressed genes: DEGs

Gene Ontology: GO

Kyoto Encyclopedia of Genes and Genomes: KEGG

protein-protein interaction: PPI

extracellular matrix: ECM

Declarations

Available of data and methods

All data generated or analyzed during the present study are included in this published article.

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Contributors

YW, BL, JL conceived this study; YW, XL, BL, PC performed this analysis; YW, BL, YJ prepared this manuscript. All authors have read and approved the submitted manuscript.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

No competing financial interests exist.

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Figures

Figure 1

The volcano plot and heatmap of DEGs. (a) The volcano plot of DEGs. The red nodes indicate the upregulated DEGs. The blue nodes indicate the downregulated DEGs. The top5 genes in both two groups were marked with names. (b) The heatmap of top100 DEGs. The expression level of top50 down-regulated and top50 up-regulated DEGs in OD and healthy controls are shown. Blue indicates low expression, and red indicates high expression.

Figure 2

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs. The color of dots indicates P. The size of dots consists with GeneRatio.

Figure 4

The complete PPI network of DEGs and two important modules. The dots of network represent the DEGs, and the lines represent the interaction between DEGs. Red stands for up-regulated genes and orange stands for down-regulated genes. (a). the complete PPI network. (b). module1. (c). module2. (d). Venn diagram of the overlapping genes according to 5 algorithms. 5 kinds of color represented 5 algorithms.

Figure 5

The proportion of infiltrating immune cells in two groups. Blue and yellow stand for healthy and Ollier chondrosarcoma respectively. (** indicates significant difference)