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Molecular changes of mesenchymal stromal cells in response to dexamethasone treatment

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Abstract. – BACKGROUND: Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of cell types. The MSCs can be activated and mobilized if needed.

AIM: This study aimed to investigate the response mechanism of MSCs under Dexamethasone (Dex) treatment by combining MSCs microarray and bioinformatics methods.

MATERIALS AND METHODS: We downloaded the gene expression profile of rat's MSCs challenge with or without Dex (GSE3339) from Gene Expression Omnibus database, including 2 Dex treated samples and 3 untreated samples. The differentially expressed genes (DEGs) were identified by packages in R language. Then, Gestalt (Genomic Sequence Total Analysis and Lookup Tool) and EASE (Expression Analysis Systematic Explorer) to were employed to obtain the molecular events of MSCs under Dex treatment.

RESULTS: A total of 17 genes were identified as DEGs between untreated and treated samples, and they were significant enriched in immune response and cell differentiation. The C3 gene was the common candidate gene selected from two different algorithms, and 24 conserved sites were identified in the 3'UTR of C3 gene.

CONCLUSIONS: Genes associated with immune response and cell differentiation were dysregulated in MSCs under Dex.

Key Words:
Mesenchymal stromal cell, Dexamethasone, Differentially expressed gene, Enrichment analysis, Conservation sites.

Instruction

Mesenchymal stem cells (MSCs), are multipotent stromal cells that can differentiate into connective and hematopoietic cells, such as chondrocytes (cartilage cells), osteoblasts (bone cells) and adipocytes (fat cells)[1,2]. The mesenchymal stem cells can be activated and mobilized if needed. Therefore, they are widely applied in clinical, such as repair of impairment of bone, cartilage, tendons and other tissues[3]. Research on the MSCs culture, proliferation and differentiation under regulation was critical.

Dexamethasone (Dex) is a potent synthetic member of the glucocorticoid class of steroid drugs that has immunosuppressant and anti-inflammatory properties[4]. It has been widely used as anti-inflammatory and chemotherapeutic agents[5]; however, prolonged use of Dex enhances direct responsiveness of osteoblasts and ultimately impairs bone formation, leading to changes in cell number and function[6]. Dex is reported to promote osteogenesis in vitro, and induce the expression of osteogenic markers in MSCs[7-9]. Although Dex have been shown to have various actions in bone cells, the molecular mechanism of Dex involved in apoptosis of osteoblasts is poorly understood.

In this study, we analyzed the MSCs microarray data under Dex treatment, and identified the differentially expressed genes (DEGs) by comparing with MSCs un-treated with Dex. Further, the molecular changes of biological process were explored and the differentiation of the MSCs was analyzed. In addition, the conserved sites on 3' untranslated region of a critical DEG was analyzed.

Materials and Methods

Affymetrix Microarray Data

The transcriptional profile of GSE3339[10] was downloaded from Gene Expression Omnibus database, which was deposited by Akavia et al. Briefly, MSCs derived from rats were cultured and challenged with 10^{-8} M Dex (n= 2). The MSCs untreated with Dex were used as control (n=3). On day 14, cells were harvested for RNA extraction and gene expression analysis. The platform was Affymetrix Rat Expression 230A

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Array (Affymetrix, Santa Clara, CA, USA), containing 15,766 probe sets and comprising 14,280 unigene clusters. We downloaded the original data and platform annotation information for further analysis.

Data Preprocessing and DEG Analysis

The original data were first preprocessed by using Affy package in R language [11, 12]. Then LIMMA package[13] was applied to compute the probability of genes being differentially expressed between MSCs challenge with or without Dex. To reduce false positive results, the BH method[14] was used to adjust the raw *p*-values into false discovery rate (FDR). The genes with FDR < 0.01 and |logFC|>1 were selected as DEGs.

Function Enrichment Analysis Based on Hypergeometric Algorithm and Fisher Exact Test

WebGestalt (http://genereg.ornl.gov/webgestalt/)[15] organizes and visualizes gene sets in various biological contexts, including Gene Ontology (GO), tissue expression pattern, chromosome distribution, metabolic and signaling pathways, protein domain information and publications by using the hypergeometric test. EASE (Expressing Analysis Systematic Explorer) is a customizable software application for rapid biological interpretation of gene lists result from high-throughput genomic data based on Fisher exact test[16].

To obtain more reliable enrichment results, we performed GO enrichment analyses used these two softwares at FDR < 0.05, respectively and screened the overlapping GO terms as final results.

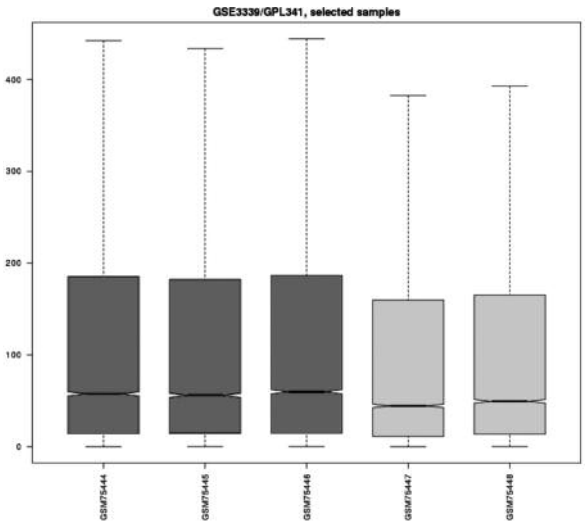

Figure 1. The standardized expression data.

Table I. The up-regulated genes (log FC > 1) and down-regulated (log FC < -1) in Dex treated cells at FDR of 0.01.

Gene	*p* value	FDR	logFC
PTPRC	4.94E-06	0.00928	-6.97267
PTPRJ	4.71E-06	0.00928	-6.88779
RT1-BA	7.15E-06	0.00928	-6.40783
RT1-DB1	2.93E-06	0.00928	-6.16392
MMP7	2.07E-06	0.00928	-5.97016
CXCL13	8.97E-06	0.00928	-5.20663
SLC17A3	7.55E-06	0.00928	-4.25691
CTSC	1.05E-05	0.00928	-4.21801
IGSF6	9.5E-06	0.00928	-3.91022
ARHGAP4	6.84E-06	0.00928	-3.77312
PIP4K2B	7.13E-06	0.00928	3.644385
ATP6V1C2	1.03E-05	0.00928	3.893746
C3	7.46E-06	0.00928	3.980625
SECTM1B	8.82E-06	0.00928	4.86468
LOC686432	1.29E-05	0.00997	4.920804
METRN	1.69E-06	0.00928	5.242093
RRAD	7.71E-06	0.00928	6.370492

Selection of 3'UTR Conserved Sites

3'-Untranslated regions (UTRs) are known to play crucial roles in the post-transcriptional regulation of gene expression, including modulation of the transport of mRNAs out of the nucleus and of translation efficiency[17], subcellular localization[18] and stability[19]. The sequences of UTR are usually conserved and similar with each other among different species. In this work, we searched the conserved sites on 3' UTR of selected DEG. The sequence information of the selected gene in 23 species were downloaded from NCBI (National Center for Biotechnology Information) public database (http://www.ncbi.nlm.nih.gov/) and the 34bp length 3'UTRs among 23 species were compared by using ClustalW[20] (http://bips.u-strasbg.fr/fr/Documentation/ClustalW/). Finally, the conserved sites of the gene in 23 species were obtained.

Results

Screening of DEGs

After data preprocessing, the quality of gene expression was significantly increased (Figure 1). At a FDR of 0.01 and |logFC| > 1, a total of 17 genes were differentially expressed between Dex treated cells and Dex untreated cells. Among them, 10 genes were down-regulated in Dex treated cells and the remaining 7 genes were up-regulated (Table I).

Table II. Gene Ontology enrichment analysis using WebGestalt (FDR < 0.05).

GO ID	Function	FDR	Gene
GO:0006955	immune response	0.0002	*CXCL13, RT1-BA, C3, RT1-DB1*
GO:0002376	immune system process	0.0014	*PTPRC,CXCL13, SECTM1B, RT1-BA,C3*
GO:0045595	regulation of cell differentiation	0.0126	*PTPRC, METRN, RT1-BA, ARHGAP4*
GO:0050793	regulation of developmental process	0.0290	*PTPRC, METRN, RT1-BA, ARHGAP4*

Function Enrichment Analysis

To identify the function of DEGs in Dex treated cells, GO enrichment analysis was performed by using WebGestalt and EASE, respectively. Based on the hypergeometric algorithm in WebGestalt, 4 GO categories were significantly enriched, including immune response, regulation of cell differentiation and regulation of developmental process (Table II). EASE based on Fisher exact test obtained 6 GO categories, including immune response, MHC class II protein complex and positive regulation of B cell mediated immunity (Table III). By combining Table II and Table III, we could find that the GO category of "immune response" was significantly enriched by both WebGestalt and EASE. By comparing the DEGs enriched in "immune response", we found that *C3* (Complement component 3) appeared in both terms and therefore, we selected *C3* for further analysis.

3'UTR Conserved Site Screening

The gene sequences of *C3* in 23 species were downloaded and compared (Figure 3). From Figure 3, we could find that 24 sites were highly conserved in the 23 species (Capital letters marked in green).

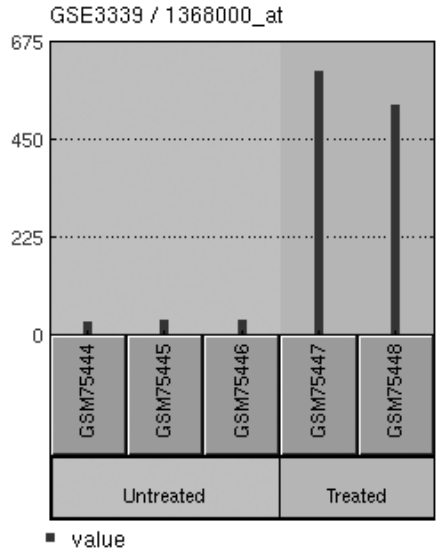

Figure 2. C3 gene expression values in different samples. Blue region is the samples with no Dexamethasone treatment, and the pink region is the samples after Dexamethasone treatment. The horizontal ordinate is the group, and the vertical coordinate is expression value. The red columns are the expression value.

Discussion

Dex is a potent synthetic member of the glucocorticoid class of steroid drugs which acts as an anti-inflammatory and immunosuppressant. It has been widely used to treat inflammatory and autoimmune conditions, such as rheumatoid arthritis and acute asthma [5, 21]. In this study, we selected a set of the specific DEGs under Dex treatment. Function enrichment analyses suggest these genes were significantly associated with immune response. By comparing the results of different al-

Table III. Gene Ontology enrichment analysis using EASE (FDR < 0.01).

GO ID	Function	FDR	Genes
GO:0006955	immune response	7.12E-05	PTPRC, C3, SECTM1B
GO:0002252	immune effector process	0.008354	PTPRC, C3, RT1-BA
GO:0050778	positive regulation of immune response	0.011208	PTPRC, C3, RT1-BA
GO:0042613	MHC class II protein complex	0.012004	RT1-DB1, RT1-BA
GO:0002891	positive regulation of immunoglobulin mediated immune response	0.014955	PTPRC, C3
GO:0002714	positive regulation of B cell mediated immunity	0.014955	PTPRC, C3

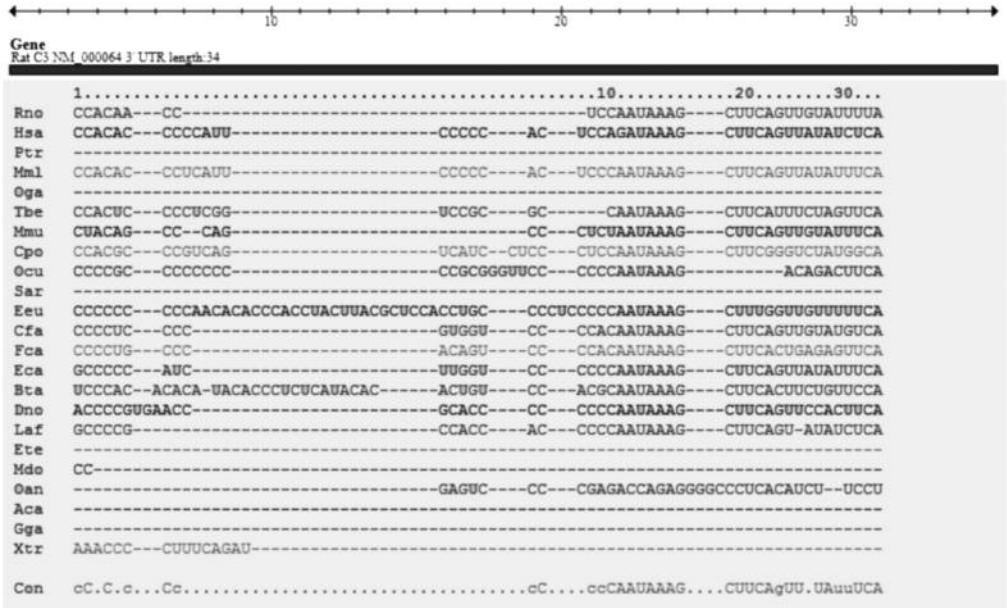

Figure 3. The 3'UTR regions of C3 gene in different species The bottom green capital font represents the high matching sites, and the lower case letter represents the low matching sites.

gorithms, we finally identified *C3* gene as the most associated gene with immune response. From Table I, we could find that C3 was significantly up-regulated under Dex treatment (FDR = 0.00928, log FC = 3.98).

C3 is a protein of the immune system. It plays a central role in the activation of complement system[22] and contributes to innate immunity. Recent works has suggested that monocyte-derived C3 is critical in the reticuloendothelial system in the local immune response[23]. This constitutes "local" complement synthesis in turn. The *in vivo* role of monocyte-derived complement, specifically C3, has been studied much less. Similar to those we have employed, researches of C3KOIR/WT chimeras have suggested a role for local (presumably monocyte/macrophage-derived) C3 in the reticuloendothelial system in the immune response to viruses[23]. As is the case in ours, this study also showed evidence of some low-level C3 present in plasma. Other reports have conversely suggested a role in the differentiation of monocytes into dendritic cells for C3 gene[24].

In earlier studies, glucocorticoids were reported to stimulate synthesis of the second component of complement (C2), factor B and C1 inhibitor[25,26]. Coulpier et al[27] examined the combined effect of IL-1α and glucocorticoids on C3 and factor B expression by the endothelial cells and observed significant potentialization of IL-1α induced stimulation of C3 and factor B production. Besides, Zach et al[28] suggested Dex enhances the expression of C3 mRNA and increase the production of functionally active C3 by A549 cells by a mechanism that is mediated by the intracellular glucocorticoid receptor. In line with previous researches, our study suggests Dex upregulated the expression of *C3* in MSCs. Combined with all these evidences, it is safe for us to infer that the regulation of *C3* expression reflect the directly differentiation of MSCs.

In addition, we identified 24 conserved sites on 3'UTR of *C3* from the view of gene sequence of 23 specifies. These conserved non-coding sequences are sufficient for subcellular localization of mRNAs[29]. It is promising to direct MSCs differentiation by drug or chemical reagent stimulation on these non-coding region sites.

Conclusions

We herein show that treatment of dexamethasone on MSCs induces immune responses behavior and immune system alterations. Additional studies suggest that a set of differentially expressed genes such as C3 gene are the precise mediators of such interplay. In addition, a total of 24 highly conserved sites on 3'UTR of C3 were selected out from the view of 23 specifies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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