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Pathway enrichment analysis of human osteosarcoma U-2 OS bone cells expose to dexamethasone

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Abstract. – OBJECTIVE: Osteosarcoma is the second highest cause of cancer-related death in children, mainly due to development of often fatal metastasis, usually in the lungs. Glucocorticoids play an important role in the treatment of a number of inflammatory diseases and immune diseases. The objective of this study was to explore the molecular mechanism of osteosarcoma in response to dexamethasone (DEX, a kind of synthetic glucocorticoid), with a view to obtain information on the pathways activated by DEX.

MATERIALS AND METHODS: By using the GSE6711 Affymetrix microarray data accessible from Gene Expression Omnibus database, we first identified the differentially expressed genes (DEGs) among different time course treatment with dexamethasone of each isoform, and the DEGs among cells expressing different GR isoforms, followed by the pathway enrichment analysis of the DEGs.

RESULTS: The results indicated that DEX could inhibit osteosarcoma cell proliferation and promote osteosarcoma cell apoptosis through induction of lots of related genes expression at the transcription level.

CONCLUSIONS: Our data provide a comprehensive bioinformatics analysis of pathways which may be involved in the response to glucocorticoids.

Key Words:
Osteosarcoma; Pathway enrichment; Dexamethasone; U-2 OS bone cells.

Introduction

Osteosarcoma (OS) is the most common histological form of primary bone cancer, and the sixth most common type of cancer in children under age 15[1]. It is the second highest cause of cancer-related death in these age groups, mainly due to development of often fatal metastasis, usually in the lungs. Despite sophisticated therapies, such as the surgery, chemotherapy, and radiotherapy have been carried out to treat patients with OS, survival for these patients is still poor and the mortality remains about 50%[2]. Non-transformed osteoblasts are highly sensitive to glucocorticoids which reduce proliferation and induce apoptosis. Previous studies suggest that OS cells can express 11 beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2) which is responsible for the interconversion of hormonally active cortisol to cortisone[3]. 11β-HSD2 is, thus, likely to contribute to the rapid proliferation of OS cells. However, OS cells can selectively activate certain synthetic glucocorticoids[4]. This indicates that targeting of cytotoxic medications to OS tissue via the expression of 11β-HSD2 has potential as a novel treatment for this disease.

Dexamethasone (DEX) is a kind of synthetic glucocorticoid, and has been reported to be activated by 11β-HSD2. Glucocorticoids (also known as corticosteroids or steroids) are the most effective anti-inflammatory treatments available for many inflammatory and immune diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease, cancer and autoimmune diseases[5-7]. Glucocorticoid action is predominantly mediated through the classic glucocorticoid receptor (GR)α isoform which represses expression of various genes encoding inflammatory mediators[8]. Besides, another isoform termed GR-β which is deficient in hormone binding has been isolated in humans[9]. Both α and β variants are generated via alternative splicing and diverge at their carboxy-termini, and GR-α isoform is expressed at relatively higher levels in the majority of the examined tissues[10]. The GR-α and GR-β isoforms generate additional isoforms via alternative translation initiation mechanisms[11,12].

The recent development of bioinformatics, an application of computer science and information technology to the field of biology and medicine,

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has been used in analyzing genes related with osteosarcoma [13]. In this present study, we focus on the pathways involved in the response to glucocorticoid in human OS cells expressing different GR isoforms. We first identified the differentially expressed genes among different time course treatment with dexamethasone of each isoform, and the differentially expressed genes among cells expressing different GR isoforms, then performed the pathway enrichment analysis of the DEGs. We aimed to explore the molecular mechanism of OS response to DEX with a view to obtain information on the pathways activated by DEX. We anticipate our study could pave the way for further DEX therapy study.

Materials and Methods

Affymetrix Microarray Data

The transcription profile of GSE6711[14,15] was downloaded from GEO (http://www.ncbi.nlm.nih.gov/geo/), a public functional genomics data repository, which is based on the Affymetrix GPL1708 platform data (Affymetrix Human Genome U95 Version 2 Array). A total of 60 chips were used for analyses. Dexamethasone-treated (n = 3 replicates of each time points -6 h, 12 h and 24 h) and untreated control (n = 3) cells were used to study human osteosarcoma U-2 OS bone cell lines exposed to DEX. Five isoforms of GR was used: -alpha, -A, -B, -C, -D. Consequently, yielding 60 chips were available in total: five isoforms by three biological replicates by four time points.

Pathway Data

Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/) is a collection of on line databases dealing with genomes, enzymatic pathways, and biological chemicals[16]. The 'pathway' database records networks of molecular interactions in the cells, and variants of them specific to particular organisms. A total of 130 pathways, involving 2287 genes, were collected from the KEGG database (updated at 2011. 06).

Differentially Expressed Genes (DEGs) Analysis

For the GSE6711 dataset, the limma[17] package in R [18] was used to identify DEGs. The original expression datasets from all conditions were extracted into expression estimates using the Robust Multiarray Average (RMA) method [19] with the default settings implemented in Bioconductor, and then the linear model was constructed. Significance of gene expression differences were tested with the classical t-test method. To circumvent the multi-test problem which might induce too much false positive results, the Benjamini and Hochberg method[20] was used to adjust the raw P-values into false discovery rate (FDR). FDR-corrected p values < 0.05 were considered statistically significant.

Pathway Enrichment Analysis

In order to facilitate the functional annotation and analysis of large lists of genes in our result, DAVID [21] (The **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery) bioinformatics resources were used. DAVID consists of an integrated biological knowledgebase and analytic tools aimed at systematically extracting biological meaning from large gene/protein lists. DAVID was used to identify over-represented KEGG pathway. The enrichment pathways with the p-value less than 0.1 and at least two genes involved the pathways were set as the threshold for clustering genes based on the hypergeometric distribution.

Results

Differentially Expressed Genes Selection

In order to get differentially expressed genes among the different time course treatments with DEX of different GR isoforms (-alpha, -A, -B, -C, -D), we obtained publicly available microarray dataset GSE6711 from GEO. A total of 60 samples were used to further analysis. The DEGs only with FDR less than 0.05 were selected. Total 2808 overlapping DEGs were identified in GR-A isoform-expressing cells, 1541 DEGs were identified in GR-B isoform-expressing cells, 3601 DEGs were identified in GR-C isoform-expressing cells and 1107 DEGs were identified in GR-D isoform-expressing cells (Figure 1). The up- or down- regulated genes were presented in additional file 1.

Different translational GR isoforms can elicit distinct glucocorticoid responses to DEX[14]. Cells expressing the GR-C isoform initiated the cell death progress as early as 12 h after DEX administration, whereas cells expressing other GR isoforms did not do so until approximately 20 h after DEX exposure. Therefore, to explore the mol-

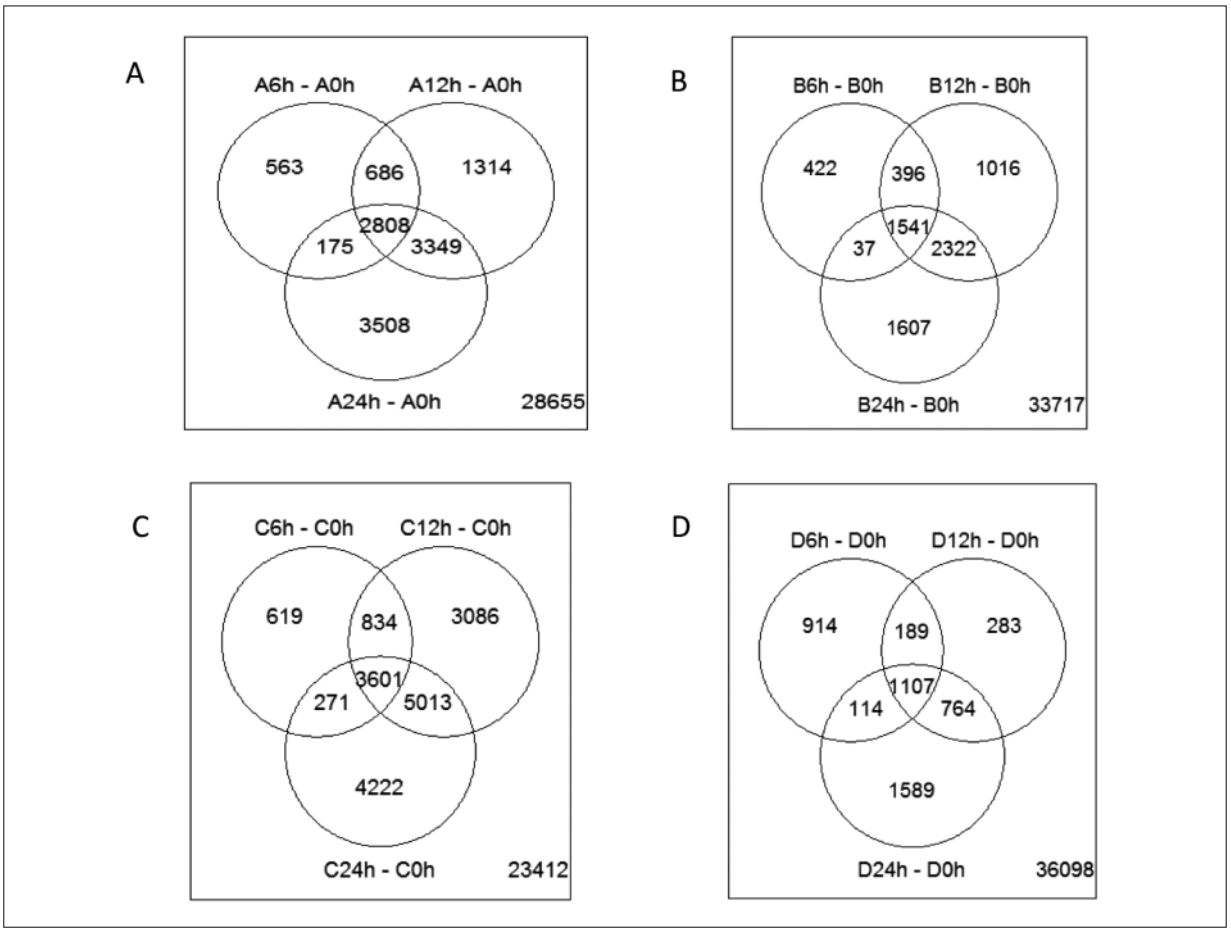

Figure 1. VENN graph showing overlap of genes differentially expressed after treatments with DEX of different glucocorticoid receptor isoforms for 6h, 12h, and 24h. $p < 0.05$ was considered statistically significant. The differential genes include both up- or down-regulated genes. ***A,*** The number of DEGs among different time course treatments with DEX of GR-A isoform-expressing cells. A total of 2808 overlapping DEGs were identified in GR-A isoform-expressing cells at three time courses. ***B,*** The number of DEGs among different time course treatments with DEX of GR-B isoform-expressing cells. A total of 1541 overlapping DEGs were identified in GR-B isoform-expressing cells. ***C,*** The number of DEGs among different time course treatments with DEX of GR-C isoform- expressing cells. A total of 3601 overlapping DEGs were identified in GR-C isoform-expressing cells. ***D,*** The number of DEGs among different time course treatments with DEX of GR-D isoform- expressing cells. A total of 1107 overlapping DEGs were identified in GR-D isoform- expressing cells. The numbers of genes that are not significantly different are 28655, 33717, 23412, and 36098, respectively.

ecular mechanisms underlying the selective transcriptional regulation of apoptotic genes in cells expressing different GR isoforms, we identified the DEGs between cells expressing GR-C and cells expressing the other GR isoforms in 6 h and 12 h samples. We got 827 DEGs between GR-A cell and GR-C cell, 1661 DEGs between GR-B and GR-C cell, and 4287 DEGs between GR-C cell and GR-D cell in 6h samples. A total of 2624 DEGs between GR-A cell and GR-C cell, 5494 DEGs between GR-B cell and GR-C cell, and 9511 DEGs between GR-C cell and GR-D cell were identified in 12h samples. Lu et al[14] also observed that cells expressing the GR-D isoform had significantly less cell death than did cells expressing any of the other GR isoforms. So we identified the DEGs between cells expressing GR-D and cells expressing the other GR isoforms. A total of 3670 DEGs between GR-A cell and GR-D cell, and 1616 DEGs between GR-B cell and GR-D cell were obtained.

Pathway Enrichment Analysis of DEGs Between Untreated and Treated with DEX

No matter which GR isoforms the cells expressing, the gene expression profile changed obviously after treated with DEX. To analyze the

significant pathways involved in the treatment, we selected the top 500 DEGs between untreated and treated with DEX for 6 h, 12 h and 24 h to perform KEGG pathway enrichment analysis. The p-value less than 0.1 and at least two genes involved the pathways were chosen as cut-off criterion. A total of 24 pathways were enriched including "calcium signaling pathway", "chronic myeloid leukemia", "steroid hormone biosynthesis", "p53 signaling pathway", "gap junction" and so on (Table I).

Pathway Enrichment Analysis of DEGs Among Different GR Isoform-Expressing cells After Treat with DEX

Because of the different responses to DEX of cells expressing different GR isoforms, the GR target genes were selectively regulated by the GR isoforms. Each GR isoform regulated a unique set of genes in addition to the commonly regulated genes[14]. Only cells expressing GR-C response to DEX whereas cells expressing other GR isoforms did not do so at 12 h after DEX administration. Therefore, we chose *p*-value less than 0.1 as threshold to identify differentially expressed genes among different GR isoforms in 12 h sample, and performed pathway enrichment analysis. Several pathways were enriched in each class (Table II). As shown in Table II, the different pathways between cells expressing GR-C and cells expressing GR-A were enriched in "oocyte meiosis", "GnRH signaling pathway", "Chemokine signaling pathway" and so on. The different pathways between cells expressing GR-C and cells expressing GR-B were enriched in "pathways in cancer", "p53 signaling pathway", "lysine degradation" and so on. The different pathways between cells expressing GR-C and cells expressing GR-D were enriched in "cell cycle", "adherens junction", "B cell receptor signaling pathway" and so on.

The GR-D isoform-expressing cells were relatively resistant to the apoptosis-inducing actions of glucocorticoid. The GR-D isoform reduced U-2 OS cell-killing capability and it maintained the NF-κB-repressing activity. Therefore, we performed pathway enrichment analysis of DEGs between cells expressing GR-D and cells expressing the other GR isoforms (Table III). As shown in Table III, the different pathways between GR-D isoform-expressing cells and GR-alpha isoform-expressing cells were enriched in "ubiquitin mediated proteolysis", "endocytosis", "chronic myeloid leukemia" and so on. The different pathways between GR-D isoform-expressing cells and GR-A isoform-expressing cells were enriched in "p53

Table I. Pathway enrichment analysis in different GR isoforms between untreated and treated with DEX.

Class	Term	Description	*p*-value
GR-alpha	hsa04020	Calcium signaling pathway	0.050952
GR-alpha	hsa04530	Tight junction	0.0902082
GR-A	hsa05220	Chronic myeloid leukemia	0.0058521
GR-A	hsa04340	Hedgehog signaling pathway	0.0340802
GR-A	hsa00604	Glycosphingolipid biosynthesis	0.0418826
GR-A	hsa02010	ABC transporters	0.0718407
GR-A	hsa04660	T cell receptor signaling pathway	0.0888133
GR-B	hsa00140	Steroid hormone biosynthesis	0.0200855
GR-B	hsa04270	Vascular smooth muscle contraction	0.0419332
GR-B	hsa00532	Chondroitin sulfate biosynthesis	0.0886472
GR-B	hsa05220	Chronic myeloid leukemia	0.0911073
GR-C	hsa04020	Calcium signaling pathway	0.0468661
GR-C	hsa00071	Fatty acid metabolism	0.0620923
GR-C	hsa04115	p53 signaling pathway	0.0687314
GR-C	hsa05220	Chronic myeloid leukemia	0.0911073
GR-C	hsa00910	Nitrogen metabolism	0.0956908
GR-D	hsa04540	Gap junction	0.0067605
GR-D	hsa04960	Aldosterone-regulated sodium reabsorption	0.0188983
GR-D	hsa00140	Steroid hormone biosynthesis	0.0276738
GR-D	hsa04510	Focal adhesion	0.0292721
GR-D	hsa04810	Regulation of actin cytoskeleton	0.0434312
GR-D	hsa05222	Small cell lung cancer	0.059582
GR-D	hsa04010	MAPK signaling pathway	0.0718238
GR-D	hsa05214	Glioma	0.0732007

Table II. Pathway enrichment analysis of DEGs between GR-C and the other GR isoforms in 12h samples.

Class	Term	Description	*p*-value
C_diffs_A_12h	hsa04114	Oocyte meiosis	0.007662
C_diffs_A_12h	hsa04912	GnRH signaling pathway	0.009247
C_diffs_A_12h	hsa05110	Vibrio cholerae infection	0.01659
C_diffs_A_12h	hsa04062	Chemokine signaling pathway	0.020352
C_diffs_A_12h	hsa04720	Long-term potentiation	0.031708
C_diffs_A_12h	hsa03410	Base excision repair	0.032668
C_diffs_A_12h	hsa00910	Nitrogen metabolism	0.033918
C_diffs_A_12h	hsa00920	Sulfur metabolism	0.034824
C_diffs_A_12h	hsa04916	Melanogenesis	0.040181
C_diffs_A_12h	hsa03018	RNA degradation	0.043313
C_diffs_A_12h	hsa00030	Pentose phosphate pathway	0.049332
C_diffs_A_12h	hsa04360	Axon guidance	0.069515
C_diffs_A_12h	hsa05211	Renal cell carcinoma	0.077493
C_diffs_A_12h	hsa04722	Neurotrophin signaling pathway	0.082848
C_diffs_A_12h	hsa05212	Pancreatic cancer	0.09175
C_diffs_A_12h	hsa03030	DNA replication	0.093223
C_diffs_B_12h	hsa05200	Pathways in cancer	7.52E-04
C_diffs_B_12h	hsa04115	p53 signaling pathway	0.001126
C_diffs_B_12h	hsa00310	Lysine degradation	0.003744
C_diffs_B_12h	hsa00250	Alanine, aspartate and glutamate metabolism	0.004792
C_diffs_B_12h	hsa04621	NOD-like receptor signaling pathway	0.008195
C_diffs_B_12h	hsa05211	Renal cell carcinoma	0.008744
C_diffs_B_12h	hsa05220	Chronic myeloid leukemia	0.010695
C_diffs_B_12h	hsa04370	VEGF signaling pathway	0.010695
C_diffs_B_12h	hsa05212	Pancreatic cancer	0.012584
C_diffs_B_12h	hsa04520	Adherens junction	0.015033
C_diffs_B_12h	hsa05214	Glioma	0.020573
C_diffs_B_12h	hsa05210	Colorectal cancer	0.023779
C_diffs_B_12h	hsa04062	Chemokine signaling pathway	0.027005
C_diffs_B_12h	hsa00910	Nitrogen metabolism	0.029215
C_diffs_B_12h	hsa00562	Inositol phosphate metabolism	0.033859
C_diffs_B_12h	hsa04666	Fc gamma R-mediated phagocytosis	0.034422
C_diffs_B_12h	hsa00020	Citrate cycle (TCA cycle)	0.035161
C_diffs_B_12h	hsa03410	Base excision repair	0.037034
C_diffs_B_12h	hsa04662	B cell receptor signaling pathway	0.038087
C_diffs_B_12h	hsa05222	Small cell lung cancer	0.041901
C_diffs_B_12h	hsa04144	Endocytosis	0.045055
C_diffs_B_12h	hsa04360	Axon guidance	0.054183
C_diffs_B_12h	hsa04070	Phosphatidylinositol signaling system	0.058254
C_diffs_B_12h	hsa03040	Spliceosome	0.06178
C_diffs_B_12h	hsa04910	Insulin signaling pathway	0.063227
C_diffs_B_12h	hsa05221	Acute myeloid leukemia	0.063637
C_diffs_B_12h	hsa05223	Non-small cell lung cancer	0.064747
C_diffs_B_12h	hsa04510	Focal adhesion	0.083686
C_diffs_B_12h	hsa05213	Endometrial cancer	0.088126
C_diffs_B_12h	hsa03440	Homologous recombination	0.095424
C_diffs_B_12h	hsa05130	Pathogenic Escherichia coli infection	0.098084
C_diffs_D_12h	hsa01040	Biosynthesis of unsaturated fatty acids	2.40E-04
C_diffs_D_12h	hsa04110	Cell cycle	0.001474
C_diffs_D_12h	hsa04520	Adherens junction	0.001881
C_diffs_D_12h	hsa04722	Neurotrophin signaling pathway	0.0021
C_diffs_D_12h	hsa00270	Cysteine and methionine metabolism	0.002645
C_diffs_D_12h	hsa00310	Lysine degradation	0.004085
C_diffs_D_12h	hsa04662	B cell receptor signaling pathway	0.00434
C_diffs_D_12h	hsa04115	p53 signaling pathway	0.004685
C_diffs_D_12h	hsa04114	Oocyte meiosis	0.004745
C_diffs_D_12h	hsa04530	Tight junction	0.006053
C_diffs_D_12h	hsa05220	Chronic myeloid leukemia	0.008444

Table continued

Table II *(Contienued)*. Pathway enrichment analysis of DEGs between GR-C and the other GR isoforms in 12h samples.

Class	Term	Description	*p*-value
C_diffs_D_12h	hsa04144	Endocytosis	0.022749
C_diffs_D_12h	hsa04370	VEGF signaling pathway	0.027612
C_diffs_D_12h	hsa04720	Long-term potentiation	0.031682
C_diffs_D_12h	hsa05214	Glioma	0.032192
C_diffs_D_12h	hsa04621	NOD-like receptor signaling pathway	0.045274
C_diffs_D_12h	hsa05130	Pathogenic Escherichia coli infection	0.046287
C_diffs_D_12h	hsa04150	mTOR signaling pathway	0.047078
C_diffs_D_12h	hsa05211	Renal cell carcinoma	0.048057
C_diffs_D_12h	hsa00400	Phenylalanine, tyrosine and tryptophan biosynthesis	0.049081
C_diffs_D_12h	hsa05200	Pathways in cancer	0.050986
C_diffs_D_12h	hsa04914	Progesterone-mediated oocyte maturation	0.051623
C_diffs_D_12h	hsa00620	Pyruvate metabolism	0.054163
C_diffs_D_12h	hsa05215	Prostate cancer	0.055039
C_diffs_D_12h	hsa00910	Nitrogen metabolism	0.056284
C_diffs_D_12h	hsa05222	Small cell lung cancer	0.057496
C_diffs_D_12h	hsa04660	T cell receptor signaling pathway	0.059254
C_diffs_D_12h	hsa04910	Insulin signaling pathway	0.061017
C_diffs_D_12h	hsa00450	Selenoamino acid metabolism	0.068441
C_diffs_D_12h	hsa00250	Alanine, aspartate and glutamate metabolism	0.070085
C_diffs_D_12h	hsa04916	Melanogenesis	0.075892
C_diffs_D_12h	hsa00600	Sphingolipid metabolism	0.079185
C_diffs_D_12h	hsa04360	Axon guidance	0.080034
C_diffs_D_12h	hsa04310	Wnt signaling pathway	0.082493
C_diffs_D_12h	hsa05219	Bladder cancer	0.087844
C_diffs_D_12h	hsa00640	Propanoate metabolism	0.091681

signaling pathway", "focal adhesion", "glioma" and so on. The different pathways between GR-D isoform-expressing cells and GR-B isoform-expressing cells were enriched in "ribosome", "nitrogen metabolism", "Wnt signaling pathway" and so on. The different pathways between GR-D isoform-expressing cells and GR-C expressing cells were shown in Table II.

Discussion

Glucocorticoids are widely used for the suppression of inflammation in chronic inflammatory diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases. They are often the most effective therapies available and their uses are limited only by systemic side effects[22]. In this present study, we aimed to explore the underlying molecular mechanism of osteosarcoma in response to DEX-treatment through pathway enrichment analysis. More than 1000 overlapping DEGs were identified in each GR isoform-expressing cells. And pathway enrichment analysis revealed that DEX could inhibit osteosarcoma cell proliferation and promote osteosarcoma cell apoptosis through induction of lots of related genes expression at the transcription level.

Glucocorticoid action is predominantly mediated through the classic GR α isoform [23]. The GR-α isoform generates additional isoforms via alternative translation initiation mechanisms [11]. From our result of differentially expressed genes selection, we could find that each GR isoform regulated a set of genes except for the commonly regulated genes. That is, each GR isoform selectively regulated the glucocorticoid target genes.

After treated with DEX, many cellular signaling pathways were activated in cells no matter which GR isoform the cell expressed. Pathways involved in anti-inflammatory, immune response, cell proliferation and apoptosis were enriched in our result, such as MAPK signaling pathway, steroid hormone biosynthesis, T cell receptor signaling pathway, hedgehog signaling pathway, p53 signaling pathway and so on.

The MAPKs (Mitogen-Activated Protein Kinases) play a key role in inflammatory cell types through transducing the response from proinflammatory cytokine receptors to the transcriptional apparatus. MAPK subgroups such as JNK regulate activation of the AP-1 complex required for proinflammatory gene expression. Negative

Table III. Pathway enrichment analysis of DEGs between GR-D and the other GR isoforms.

Class	Term	Description	*p*-value
alpha_diffs_D_6h	hsa04120	Ubiquitin mediated proteolysis	5.78E-05
alpha_diffs_D_6h	hsa04144	Endocytosis	1.91E-04
alpha_diffs_D_6h	hsa05220	Chronic myeloid leukemia	4.28E-04
alpha_diffs_D_6h	hsa05200	Pathways in cancer	7.12E-04
alpha_diffs_D_6h	hsa05222	Small cell lung cancer	0.006801
alpha_diffs_D_6h	hsa04115	p53 signaling pathway	0.011148
alpha_diffs_D_6h	hsa00310	Lysine degradation	0.015512
alpha_diffs_D_6h	hsa04110	Cell cycle	0.017173
alpha_diffs_D_6h	hsa05212	Pancreatic cancer	0.022646
alpha_diffs_D_6h	hsa03022	Basal transcription factors	0.023219
alpha_diffs_D_6h	hsa00520	Amino sugar and nucleotide sugar metabolism	0.034034
alpha_diffs_D_6h	hsa05214	Glioma	0.034313
alpha_diffs_D_6h	hsa05218	Melanoma	0.035635
alpha_diffs_D_6h	hsa04722	Neurotrophin signaling pathway	0.039818
alpha_diffs_D_6h	hsa00561	Glycerolipid metabolism	0.041331
alpha_diffs_D_6h	hsa04130	SNARE interactions in vesicular transport	0.045435
alpha_diffs_D_6h	hsa04510	Focal adhesion	0.047411
alpha_diffs_D_6h	hsa05223	Non-small cell lung cancer	0.052349
alpha_diffs_D_6h	hsa05211	Renal cell carcinoma	0.054618
alpha_diffs_D_6h	hsa00600	Sphingolipid metabolism	0.055336
alpha_diffs_D_6h	hsa00640	Propanoate metabolism	0.061051
alpha_diffs_D_6h	hsa04920	Adipocytokine signaling pathway	0.062554
alpha_diffs_D_6h	hsa04960	Aldosterone-regulated sodium reabsorption	0.079281
alpha_diffs_D_6h	hsa00330	Arginine and proline metabolism	0.082033
alpha_diffs_D_6h	hsa05219	Bladder cancer	0.0934
alpha_diffs_D_6h	hsa04940	Type I diabetes mellitus	0.0934
alpha_diffs_D_6h	hsa04010	MAPK signaling pathway	0.095302
A_diffs_D_12h	hsa04115	p53 signaling pathway	0.00133
A_diffs_D_12h	hsa05322	Systemic lupus erythematosus	0.001745
A_diffs_D_12h	hsa05200	Pathways in cancer	0.005607
A_diffs_D_12h	hsa04510	Focal adhesion	0.009776
A_diffs_D_12h	hsa05214	Glioma	0.017514
A_diffs_D_12h	hsa05220	Chronic myeloid leukemia	0.021572
A_diffs_D_12h	hsa00520	Amino sugar and nucleotide sugar metabolism	0.021685
A_diffs_D_12h	hsa01040	Biosynthesis of unsaturated fatty acids	0.033187
A_diffs_D_12h	hsa05130	Pathogenic Escherichia coli infection	0.033241
A_diffs_D_12h	hsa04130	SNARE interactions in vesicular transport	0.043485
A_diffs_D_12h	hsa04530	Tight junction	0.050905
A_diffs_D_12h	hsa05217	Basal cell carcinoma	0.052006
A_diffs_D_12h	hsa04520	Adherens junction	0.052182
A_diffs_D_12h	hsa00270	Cysteine and methionine metabolism	0.052231
A_diffs_D_12h	hsa00561	Glycerolipid metabolism	0.057166
A_diffs_D_12h	hsa00240	Pyrimidine metabolism	0.057924
A_diffs_D_12h	hsa05416	Viral myocarditis	0.08973
A_diffs_D_12h	hsa05218	Melanoma	0.08973
B_diffs_D_12h	hsa03010	Ribosome	2.24E-05
B_diffs_D_12h	hsa05322	Systemic lupus erythematosus	4.18E-04
B_diffs_D_12h	hsa00910	Nitrogen metabolism	0.019049
B_diffs_D_12h	hsa04310	Wnt signaling pathway	0.032979
B_diffs_D_12h	hsa05130	Pathogenic Escherichia coli infection	0.042652
B_diffs_D_12h	hsa04540	Gap junction	0.044205
B_diffs_D_12h	hsa04722	Neurotrophin signaling pathway	0.049068
B_diffs_D_12h	hsa01040	Biosynthesis of unsaturated fatty acids	0.062836

regulation of the MAPK family by glucocorticoids may be an additional mechanism by which the GR exerts its anti-inflammatory effects [24]. The GR represses the MAPK family by inhibiting the phosphorylation step required for their activation. The defined molecular mechanism behind this inhibition has not been fully characterized and may be cell type and stimulus specific[25].

Steroid hormones are familiar clinically and physiologically as regulators of physiological processes[26]. Glucocorticoid binds GR, which is a member of the steroid receptor super-family, and activates the transcription through GRE (glucocorticoid response element)[27]. Glucocorticoids potently modulate immune function and are a mainstay of therapy for treatment of autoimmune diseases, leukemias and lymphomas[28]. Tp53 is a DNA-binding protein which responds to diverse cellular stresses to regulate target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism[29]. Our result clearly indicated the significant pathways of the GR isoforms involved after treated with DEX.

As the translationally generated GR isoforms have distinct functions in apoptosis-inducing activities, the GR-C isoform was significantly more active in doing so than any other GR isoform; we performed pathway enrichment analysis between cells expressing GR-C isoform and cells expressing other isoforms. As shown in table 2, most of the different pathways between cells expressing GR-C and cells expressing other GR isoforms were enriched in pathways related to cell apoptosis, such as "RNA degradation", "renal cell carcinoma", "chemokine signaling pathway" between cells expressing GR-A and cells expressing GR-C, "pathways in cancer", "p53 signaling pathway", "NOD-like receptor signaling pathway" between cells expressing GR-B and cells expressing GR-C, "lysine degradation", "VEGF signaling pathway" "chronic myeloid leukemia" between cells expressing GR-C and cells expressing GR-D.

NOD-like receptors, in short for nucleotide oligomerization domain receptors, are cytoplasmic proteins that may have a variety of functions in regulation of inflammatory and apoptotic responses. NOD-like receptors may recognize endogenous or microbial molecules, perhaps including molecules produced in response to stress. They may then form oligomers that activate inflammatory caspases (e.g. caspase1), causing cleavage and activation of important inflammatory cytokines such as IL-1. NLRs may also activate the NF- B signaling pathway to induce production of inflammatory molecules[30-32].

VEGF (Vascular Endothelial Growth Factor) is an angiogenic factor that regulates multiple endothelial cell functions, including mitogenesis. The VEGF stimulate cellular responses by binding to tyrosine kinase receptors (the VEGFRs) on the cell surface, causing them to dimerize and become activated through transphosphorylation. It appears to play a role in the progression of acute and chronic leukemias[33].

Conclusions

We have demonstrated that DEX could inhibit osteosarcoma cell proliferation and promote osteosarcoma cell apoptosis through interaction with various isoforms of GR which induce lots of related genes expression at transcription level. Osteosarcoma cells expressing different GR isoforms enriched in various pathways after treated with DEX, suggesting that the functions of glucocorticoid are differ among cells expressing different GR isoforms. Our data provide a basic understanding of molecular mechanism of the osteosarcoma response to glucocorticoid. Further analysis on the enriched pathways in our result may help pave the way for novel, safer therapies of osteosarcoma.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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