

Analysis Of Dexamethasone Effect On Gene Expression Patterns In Primary Synoviocytes: Insights Into Clinical Use

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INTRODUCTION: Corticosteroids are widely acknowledged as a first line treatment approach for chronic inflammatory disorders like osteoarthritis and spondylitis. However, their effect extends beyond the downregulation of proinflammatory pathways and immune responses. These agents also play a crucial role in tissue remodeling, and their use is a cornerstone in the management of conditions involving excessive tissue growth and fibrosis. However, their precise effect on the molecular environment of synovium remains inadequately researched. In this study, we examined the effect of subtherapeutic doses of dexamethasone on gene expression patterns in primary synoviocytes.

METHODS: The study was performed in primary human fibroblast-like synoviocytes (HFLS, Cell Applications, Inc.) and HEK293T cells (ATCC) maintained under standard conditions. HEK293T cells served as a background control to distinguish the dexamethasone-induced differential expression specific for HFLS. The cells were collected after treatment with 50 nM dexamethasone (MilliporeSigma) in DMSO for 4 h and 24 h respectively. Total RNA was isolated using the RNeasy Mini Kit (Qiagen). Libraries of cDNA for paired-end sequencing were prepared using the TruSeq Stranded mRNA-Seq Library Preparation Kit (Illumina). Samples were sequenced with Illumina HiSeq 2500 system (Illumina) and 150 base pair paired-end reads were generated. Libraries of cDNA for paired-end sequencing were prepared using the TruSeq Stranded mRNA-Seq Library Preparation Kit (Illumina). Samples were sequenced with Illumina HiSeq 2500 system (Illumina) and 125 base pair paired-end reads were generated. The quality control of raw sequence data was performed with FastQC followed by trimming of low-quality reads with Trimmomatic. Trimmed paired-end reads were aligned using Hisat2 to hg38 reference genome. Counts matrix was obtained using FeatureCounts. Differentially expressed genes were identified using the pyDESeq2 package.

RESULTS SECTION: The data analysis was performed side-by-side in HFLS and HEK293T cells treated with dexamethasone. The changes in gene expression were evaluated and compared at 4 h and 24 h after treatment. All experiments were performed in duplicates. The performed transcriptomic analysis revealed a prominence of two distinct clusters of early- and late-response genes, indicating intricate mechanisms underlying dexamethasone's effects. A specific and significant change in the expression level compared to HEK293T cells was observed for 3427 genes in HFLS cells at the 4 h time point. A specific late activation in HFLS cells was observed for 2170 genes at the 24 h timepoint. Furthermore, we demonstrate not just the anticipated downregulation of inflammation-associated genes such as *LIF* and *CXCL1*, but also the upregulation of broader gene networks such as hormone-receptor associated ones. We find particularly interesting the activation of *RXFP* gene family, which encodes different types of receptors for antifibrotic relaxin hormone. Finally, we observe the upregulation of *FKBP5*, which encodes a negative regulator of the glucocorticoid receptor and suggests the existence of the negative feedback loop for dexamethasone in HFLS cells.

DISCUSSION: The performed study constitutes a comprehensive investigation into the effects of dexamethasone on gene expression in synoviocytes. Our findings challenge the conventional view of this drug's actions as primarily anti-inflammatory, revealing its more profound influence on intracellular machinery. Furthermore, the potential modulation of cellular responsiveness through subtle adjustments in surface receptor repertoire highlights the multifaceted impact of dexamethasone. The significant upregulation of hormone receptor genes provides new insights for synergistic therapeutic strategies addressing synovium conditions rooted in inflammation.

CLINICAL RELEVANCE: The results of this study hold significance in comprehending the intricate effects of dexamethasone on synoviocytes. These insights will lay the foundation for more efficient therapies aimed at synovium-related disorders, consequently improving treatment outcomes.

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