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Overexpression of GRß in colonic mucosal cell line partly reflects altered gene expression in colonic mucosa of patients with inflammatory bowel disease

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ABSTRACT

The glucocorticoid receptor (GR) plays a crucial role in inflammatory responses. GR has several isoforms, of which the most deeply studied are the GRα and GRß. Recently it has been suggested that in addition to its negative dominant effect on GRα, the GRß may have a GRα-independent transcriptional activity. The GRß isoform was found to be frequently overexpressed in various autoimmune diseases, including inflammatory bowel disease (IBD). In this study, we wished to test whether the gene expression profile found in a GRß overexpressing intestinal cell line (Caco-2GRß) might mimic the gene expression alterations found in patients with IBD. Whole genome microarray analysis was performed in both normal and GRß overexpressing Caco-2 cell lines with and without dexamethasone treatment. IBD-related genes were identified from a meta-analysis of 245 microarrays available in online microarray deposits performed on intestinal mucosa samples from patients with IBD and healthy individuals. The differentially expressed genes were further studied using in silico pathway analysis. Overexpression of GRß altered a large proportion of genes that were not regulated by dexamethasone suggesting that GRß may have a GRα-independent role in the regulation of gene expression. About 10% of genes differentially expressed in colonic mucosa samples from IBD patients compared to normal subjects were also detected in Caco-2 GRß intestinal cell line. Common genes are involved in cell adhesion and cell proliferation. Overexpression of GRß in intestinal cells may affect appropriate mucosal repair and intact barrier function. The proposed novel role of GRß in intestinal epithelium warrants further studies.

1. Introduction

Glucocorticoids play an essential role in the regulation of inflammation, tissue destruction and repair [1,2]. Glucocorticoid action is mediated via the glucocorticoid receptor, mainly by the alpha isoform (GRα). GRα is widely expressed in human tissues and, in the absence of ligand, it is located in the cytoplasm as a component of a multi-protein complex. Upon ligand binding GRα dissociates from the heterocomplex, it forms homodimers and translocates to the nucleus, where it regulates the expression of various genes through direct binding to the DNA at glucocorticoid response elements (GRE) or through interaction with other transcription factors. The structure of GRß is identical to GRα up to the first 727 N-terminal amino acids, but as a consequence of alternative splicing GRß has an unique 728–742 amino acids C-terminal domain [3]. The GRß is unable to bind glucocorticoids, it does not induce transcription on GRE-containing promoters but it exerts a negative dominant effect on GRα function [3–5]. Some recent studies have questioned this sole role and suggested that GRß may have a GRα-independent transcriptional activity [6–8].

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The local imbalance of pro- and anti-inflammatory signals determined by the interplay of cytokines and glucocorticoids may contribute to chronic inflammation. Glucocorticoids have potent immunosuppressive effects and they exert anti-inflammatory ability mainly through transrepression of the NF-κB and AP-1 signaling pathways [9]. Pro-inflammatory cytokines cause a shift in the GRα/GRβ ratio through an increase of GRß that leads to glucocorticoid insensitivity [10]. GRß was frequently found to be overexpressed in various autoimmune conditions (for review see Szappanos et al. [2]). Increased GRß expression was detected in peripheral blood mononuclear cells and colonic mucosa in inflammatory bowel disease (IBD) and was linked to glucocorticoid insensitivity [11–13].

IBD is characterized by chronic inflammation of the gastrointestinal tract. Based on clinical and histological observations the two types of IBD are Crohn's disease (CD) and ulcerative colitis (UC). The pathogenesis of IBD is multifactorial; genetic susceptibility, certain environmental factors and dysregulated immune response may trigger intestinal inflammation. Epithelial barrier function is also crucial in IBD because a defective mucosal barrier fails to prevent the invasion of intestinal bacteria into deeper layers. Microbial antigens subsequently activate T-cells which contribute to tissue inflammation [14,15]. Microarray studies highlighted several differently expressed genes in IBD. Dysregulation of antimicrobial peptides has been shown in the inflamed mucosa, that was restored after infliximab therapy [15]. Inflammation related genes coding cytokines, chemokines and T-cell related transcription factors are also typically altered in IBD [14,16,17]. Furthermore, the background of increased intestinal permeability in UC includes differential expression of extracellular matrix and tight junction related genes [18] (Table 1).

In the present study we wished to characterize the effects of GRß overexpression in Caco-2 intestinal cell line. Therefore we created a stably GRß overexpressing Caco-2GRß cell line from Caco-2 cells. We evaluated the influence of increased GRß expression on the transcriptome both in the presence and absence of dexamethasone (DEX). Because GRß has been found to be upregulated in mucosal cells in IBD, we wanted to explore whether increased GRß expression may trigger archetypal gene expression alterations characteristic for IBD. Therefore, we performed a meta-analysis of the available microarray data from IBD intestinal samples and compared these results to our in vitro microarray data to identify overlaps between increased GRß expression in Caco-2GRß cells and intestinal mucosa from IBD patients.

2. Methods

2.1. Cell culture

Caco-2 and Caco-2GRß cells were grown in Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 0,1 mM non-essential amino acids and 1% penicillin/streptomycin. All compounds were purchased from Sigma-Aldrich. Cells were cultured in a humidified incubator infused with 5% CO_2 at 37C°.

2.2. Generation of a stably GRß expressing Caco-2GRß cell line

GRß was cloned from the GRα isoform using a sense oligonucleotide primer mapping to the shared α-β region and an antisense oligonucleotide specific for the GRß sequence (Fw: ATGGACTCCAAAGAATCATTAACTCC;Rev:TCAGATTAATGTGTGAGATGTGCTTTCTGGTTTTAACCACATAACATTTTCATGCATAGAATCCAAG). The PCR fragments were cloned into pcDNA3.1 vectors (Invitrogen). Sequence of plasmids was verified by direct DNA sequencing. Caco-2 cells were transfected either with the GRß containing plasmids or the empty pcDNA3.1 vector using FuGene Transfection Reagent (Promega) according to the manufacturer's instructions. Clonal selection was performed with neomycin treatment. GRα and GRβ mRNAs were quantified with Real-Time (RT) PCR. For separate detection of GRα (Genebank accession nr: X03225) and GRβ (Genebank accession nr: X03348) mRNAs, primers and probes were designed. The amplification of GRα was performed with the following primers: GRαF, 5′-AACTGGCAGCGGTTTTATCAA-3′ and GRαR, 5′- TGGAAGCAATAGT TAAGGAGATTTTCA-3′. The nucleotide sequence of the Taqman probewas: FAM-CCACTTCATGCATAGAATCCAAGAGTTTTGTCA-TAMRA. The sequences of primer pairs for the amplification of GRβ were: GRβF, 5′- AACTGGCAGCGGTTTTATCAA-3′ and GRβR, 5′-TGTGAGATGTGCTTTCTGG TTTTAA-3′, and the sequence of the Taqman probe was FAM-CATAACATTTTCATGCATAGAATCCAAGAGTTTTGTCA-TAMRA. All primer pairs were synthesized by Genosys, Sigma (Oakville, Canada) and were labelled with FAM and TAMRA [19].

Table 1
Microarray studies used in our meta-analysis. Microarray deposits of colonic samples from IBD and healthy patients retrieved from GEO and ArrayExpress databases. All studies were performed on Affimetrix GeneChip Human Genome U133 Plus 2.0 arrays.

Author	Title	Control	Crohn's disease	Ulcerative colitis	GEO/array express accession number	PMID
Ahrens	Intestinal macrophage/epithelial cell-derived CCL11/eotaxin-1 mediates eosinophil recruitment and function in pediatric ulcerative colitis [33]	4	0	8	GSE10191	18981162
Arijs	Mucosal gene expression of antimicrobial peptides in inflammatory bowel disease before and after first infliximab treatment [15]	6	19	24	GSE16879	19956723
Bjerrum	Genome-wide gene expression analysis of mucosal colonic biopsies and isolated colonocytes suggests a continuous inflammatory state in the lamina propria of patients with quiescent ulcerative colitis [16]	10	0	17	GSE13367	19834973
Carey	Activation of an IL-6:STAT3-dependent transcriptome in pediatric-onset inflammatory bowel disease [34]	0	10	5	GSE9686	18069684
Csillag	Clinical phenotype and gene expression profile in patients with Crohn's disease [17]	1	50	0	E-TABM-118	16959948
Funke	Selective downregulation of retinoic acid-inducible gene I within the intestinal epithelial compartment in Crohn's disease [35]	3	3	6	E-MEXP-2083	21830273
Kugathasan	Loci on 20q13 and 21q22 are associated with pediatric-onset inflammatory bowel disease [36]	11	32	10	GSE10616	18758464
Olsen	Diagnosis of ulcerative colitis before onset of inflammation by multivariate modeling of genome-wide gene expression data [37]	5	0	21	GSE9452	19177426