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Heterogeneity in mechanisms influencing glucocorticoid sensitivity: The need for a systems biology approach to treatment of glucocorticoid-resistant inflammation

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ABSTRACT

Glucocorticoids (GCs) have impressive anti-inflammatory and immunosuppressive effects and show a diversity of actions across a variety of cell phenotypes. Implicit in efforts to optimize GCs as anti-inflammatory agents for any or all indications is the notion that the relevant mechanism(s) of action of GCs are fully elucidated. However, recent advances in understanding GC signalling mechanisms have revealed remarkable complexity and contextual dependence, calling into question whether the mechanisms of action are sufficiently well-described to embark on optimization. In the current review, we address evidence for differences in the mechanism of action in different cell types and contexts, and discuss contrasts in mechanisms of glucocorticoid insensitivity, with a focus on asthma and Chronic Obstructive Pulmonary Disease (COPD). Given this complexity, we consider the potential breadth of impact and selectivity of strategies directed to reversing the glucocorticoid insensitivity.

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1. Introduction

Glucocorticoids (GCs) are efficacious in the treatment of acute and chronic inflammatory conditions, certain cancers and in a range of other human diseases. The breadth of indications and the relatively high efficacy of synthetic GCs as anti-inflammatory agents have sustained deep interest in optimizing GCs for their varied indications. The effort to optimize GCs as anti-inflammatory agents for any or all

Abbreviations: (11β-HSD), 11β-hydroxysteroid dehydrogenase; (ASM), Airway smooth muscle; (COPD), Chronic Obstructive Pulmonary Disease; (EBC), Exhaled Breath Condensate; (EMT), Epithelial–Mesenchymal transition; (GC), Glucocorticoid; (GILZ), Glucocorticoid-inducible leucine zipper; (GR), Glucocorticoid Receptor; (GRE), Glucocorticoid Response Element; (HDAC), Histone deacetylase; (ICS), Inhaled corticosteroid; (IL), Interleukin; (IPF), Idiopathic pulmonary fibrosis; (JNK), c-Jun N-terminal kinase; (MSC), Mesenchymal stem cell; (MAPK), Mitogen-activated protein kinase; (MKP-1), MAPK phosphatase 1; (MMP), Matrix metalloproteinase; (NK cell), Natural killer cells; (NKT cells), Natural killer T cells; (PBMC), Peripheral blood mononuclear cell; (PNMT), Phenylethanolamine N-methyltransferase; (RSV), Respiratory syncytial virus; (UTR), Untranslated region

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indications is predicated on the notion that the mechanism(s) of action of GCs are fully elucidated. At one level these mechanistic insights may seem self-evident. The GC drug class acts at a single receptor type, the glucocorticoid receptor (GR), to modulate gene transcription. The resultant changes in levels of encoded proteins (i.e., decreased levels of pro-inflammatory proteins and increased levels of anti-inflammatory proteins) underpin the therapeutic effects. However, it is now known that remarkable molecular complexity underlies this modulation of gene transcription. As further complexity continues to be unravelled, a question therefore arises: will it ever be possible to synthesise from reductionist contexts, the various components and interactions of GC signalling mechanisms, or is a new integrative paradigm required to optimize GCs?

The growing complexity in the detail of GC signalling mechanisms is readily appreciated by considering the multiple isoforms of GR now identified (Duma et al, 2006; Lu & Cidlowski, 2006), and the multiple signalling mechanisms of GR currently described (Fig. 1) (reviewed in De Bosscher & Haegeman, 2009; Revollo & Cidlowski, 2009; Ramamoorthy & Cidlowski, 2013). Each GR signalling mechanism may occur simultaneously, and either independently or inter-dependently, resulting in broad consequences for cellular function. One may therefore begin to question whether any of the various mechanisms of action are sufficiently well-described to embark on optimization. Furthermore, one or other molecular action of GR may dominate under different cellular contexts (Fig. 2). Most cell types express functional GRs. However, different cell types exist in different microenvironments, and inherently possess different genomic contexts for gene transcription. It follows that the molecular activation of GR by GCs will differ in different cell types. There is an imperative to not only understand which cell phenotypes are responsible for the orchestration and perpetuation of chronic inflammation, but to also understand both where and how GCs are acting to produce their anti-inflammatory actions, *and* where and how GC actions are limited in a GC-resistant state.

2. Heterogeneity in molecular mechanisms of glucocorticoid action

Ligand-bound GR homodimers can interact directly with DNA at glucocorticoid response elements (GREs), both upstream and downstream of the transcription start site of target genes, resulting in increased transcription of these genes (transactivation). Monomeric ligand-bound GR can also modulate gene transcription through protein–protein interactions by binding to and reducing the transactivation activity of other transcription factors, such as NF-κB and AP-1 (transrepression). GR can also bind directly to DNA at negative GREs (nGREs) to repress gene transcription (Surjit et al., 2011). Furthermore, in certain cases, GR binding to other transcription factors may synergistically enhance the transcriptional activity of these factors (e.g., STAT3 (Langlais et al, 2012), STAT5 (Stoecklin et al, 1999), AP-1 (Wu & Bresnick, 2007), NF-κB (Altonsy et al, 2014)). The splice variant GRβ, once thought to be a non-ligand binding, dominant negative inhibitor of GRα, has more recently also been shown to have transcriptional activity of its own, regulating the expression of a distinct set of genes to that regulated by GRα (Lewis-Tuffin et al, 2007; Kino et al, 2009).

Adding to the complexity of GR signalling, alternative types of GREs have been reported following identification of a repeated half-site motif in the phenylethanolamine N-methyltransferase (PNMT) gene (Adams et al, 2003), contrasting with the classical inverted repeat (palindromic) consensus sequence originally described (Grange, Roux, Rigaud, & Pictet, 1989; Payvar, et al., 1982). The type of GRE may contribute to transcriptional selectivity, as a GR dimerisation mutant mouse strain (GR^{dim}) shows some transactivation functionality, with some genes, including PNMT and MKP-1 (DUSP1), remaining fully inducible (Reichardt et al., 1998; Rogatsky et al., 2003; Abraham et al., 2006). Similarly, mutations of conserved GRE motifs have recently been shown to lack the predicted universal effect on GR-induced gene expression (Muzikar et al., 2009). Different genes have also been shown to be differentially impacted by mutations in GR protein domains other than

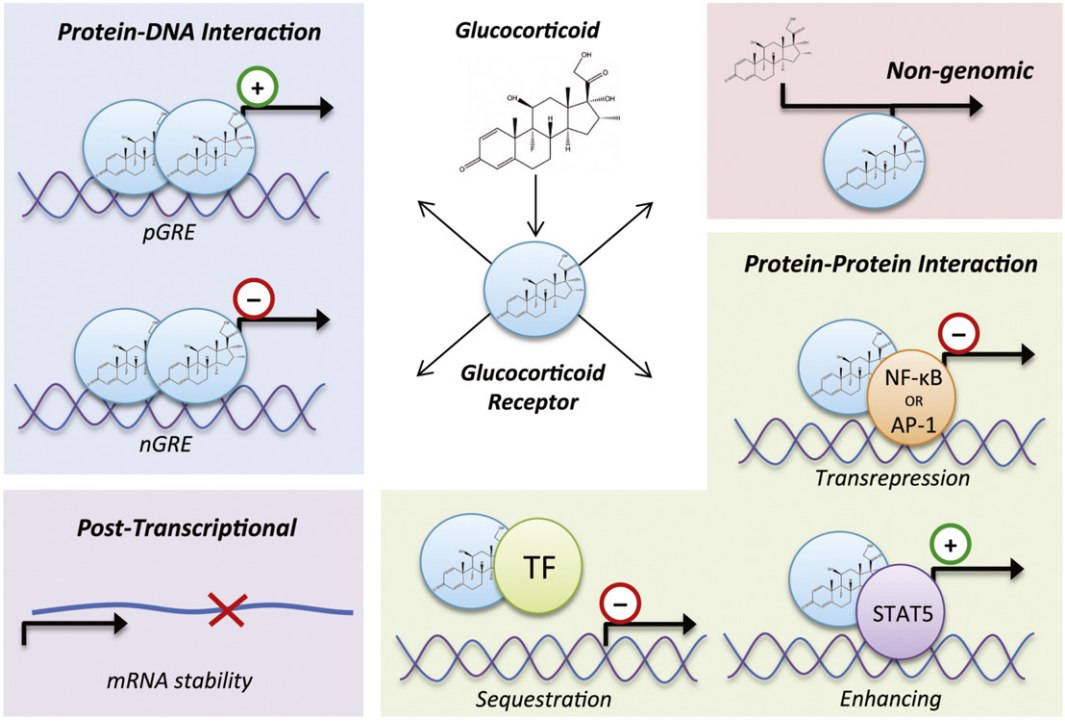

Fig. 1. Are glucocorticoid signalling mechanisms sufficiently well-described to embark on optimization? A variety of independent and inter-dependent glucocorticoid signalling mechanisms mediate the diversity of functional effects of glucocorticoid treatment. Glucocorticoids signal via the glucocorticoid receptor which can interact with DNA or other proteins to induce or inhibit gene transcription. Glucocorticoids can also modulate expression at a post-transcriptional level and can signal via rapid non-genomic pathways as well. Current understanding suggests that the cellular and genomic context of signalling may determine which molecular action may dominate in a given context.