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REVIEW ARTICLE

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Molecular pharmacology of glucocorticoids: recent advances and future perspectives

Abstract Glucocorticoids are one of the most widely used bullets for the treatment of inflammatory and immune disorders. They act by binding to their specific intracellular receptor, the glucocorticoid receptor (GR), which is a transcription factor belonging to the nuclear receptor superfamily. It is believed that the GR, upon binding ligand, elicits transcriptional regulation of target gene expression via orchestrated interaction with DNA, coregulators, other transcription factors, and chromatin. This model has raised the possibility that a certain class of ligand might variably modulate GR-mediated intracellular signals. Moreover, crystallographic analysis of the ligand-binding domain of the nuclear receptor has given structural insight into the ligand-dependent modularity of the receptor function. This advanced technology would allow the molecular pharmacologic development of a ligand that could dissociate therapeutic actions from the undesirable metabolic effects of glucocorticoids in the near future.

Key words Antiinflammation · Glucocorticoid · Glucocorticoid receptor · Immunosuppression · Transcription factor

Introduction

Glucocorticoids have been indispensable in the treatment of rheumatic diseases. However, the long-term use of glucocorticoids sometimes has undesirable effects, some of which may infringe on the quality of life of patients, as reviewed by Oshima.[1] This dilemma has prompted researchers to develop a novel glucocorticoid therapy that could dissociate the therapeutic effects from the side effects.

In the 15 years since the cDNA of the glucocorticoid receptor (GR) was cloned, the molecular biology of the action of glucocorticoid has developed rapidly and has led the way to a molecular understanding of the structure–function relationship of the GR. Based on these advances, the concept of a dissociated ligand has been clarified, as in the case of the estrogen receptor.

This review briefly summarizes recent developments in GR research and discusses the future direction of glucocorticoid therapy.

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Mechanism of glucocorticoid action

Physiological role of glucocorticoids

Glucocorticoids are formed in the zona fasciculata of the adrenal cortex and secreted into the bloodstream in response to the activity of the hypothalamic–pituitary–adrenal axis. They are indispensable to mammalian homeostatic regulation.[2] Glucocorticoids have an effect on every system of the body, although the name derives from their effects on carbohydrate metabolism. Because so many physiological processes are affected, it is difficult to formulate a unifying definition of glucocorticoid action.[3] Since most of the physiological actions of glucocorticoids are mediated by binding to a specific intracellular protein GR, this receptor mediation could serve as a functional definition of a glucocorticoid effect. Glucocorticoids may also exert nontranscriptional effects by binding to corticosteroid-binding globulin (CBG) that has bound to specific cell-surface CBG receptors in target tissues.[4,5] The glucocorticoid signal that finally influences gene expression is transmitted to the nucleus via the GR. Targeted disruption of the GR gene results in serious maturation defects and death soon after birth in mice.[6–8] Endogenous glucocorticoid excess suppresses immunologic responses,[9] and latent infections (e.g., tuberculosis) may be reactived by the administration of

pharmacological doses of glucocorticoids. On the other hand, the immunosuppressive properties of glucocorticoids are exploited in the treatment of autoimmune diseases and inflammatory states and in the field of organ transplantation. A variety of effects on components of the immunologic and inflammatory responses have been described in vitro and in animal models, but which phenomena are most relevant to the physiological role of glucocorticoids in immunomodulation remains to be demonstrated. Glucocorticoid actions are summarized below.

- Effects on metabolism: glycogen metabolism, glycogenesis, peripheral glucose utilization, lipid metabolism.
- Effects on musculoskeletal and connective tissues: bone and mineral metabolism, skeletal muscle, and connective tissue.
- Effects on fluid and electrolyte homeostasis.
- Neuropsychiatric and behavioral effects.
- Gastrointestinal effects.
- Developmental effects.
- Further actions: antiinflammatory and antiallergic effects.

GR as a nuclear receptor

Glucocorticoids are believed to act via binding to the GR, which is ubiquitously distributed and acts as a ligand-dependent transcription factor belonging to the superfamily of the nuclear receptors.[10,11] Nuclear receptors are still being found, and more than 300 sequences have been reported. Many are important transcriptional regulators involved in widely diverse physiological functions such as the control of embryonic development, cell differentiation, and metabolic homeostasis.[12] Because of the complexity of the nomenclature of nuclear receptors, a unified nomenclature system is currently being developed.[13] According to this system, GR, as NR3C1, belongs to the same subfamily as the receptors for mineralocorticoid, andorogen, progesterone, and estrogen (members of this subfamily are sometimes classified as type I nuclear receptors). In human GR, two isoforms, GRα and GRβ, consist of 777 and 742 amino acids, respectively, are splicing variants of the GR gene, and differ in the carboxy terminal.[14] Although several investigators have indicated that GRβ acts as a dominant negative GR, others have presented contradictory results.[15–25] Therefore, the function of GRβ should be further elucidated.

Members of the nuclear receptor superfamily, including the GR, share several structural features, e.g., the ligand-binding domain (LBD), the DNA-binding domain (DBD), and several transactivation domains (Fig. 1). The N-terminal domain (AF-1) contains sequences responsible for the activation of target genes and it presumably interacts with components of the basal transcription machinery, and/or with cofactors and other transcription factors, largely in a cell- or tissue-specific context. In the estrogen receptor (ER), this region is also known to be regulated by non-endocrine pathways, involving, for example, protein kinases, which are often responsible for cell signaling.[26–31] O'Malley's group has presented evidence showing that a particular RNA, called the steroid receptor RNA activator (SRA), selectively interacts with this AF-1 region and acts as a coactivator.[32] Recently, Kato's group discovered RNA helicase, which is a novel coactivator that bridges the ER and SRA.[33] Moreover, this region, as well as the LBD, has recently been suggested to be targeted by a member of the vitamin D receptor interacting protein (DRIP)/thyroid hormone receptor-associated protein (TRAP) cofactors.[34] The central part of the receptor is the DBD, which also participates in receptor dimerization, nuclear translocation, and transactivation.

The structural motif of the DBD is two zinc fingers formed by the coordination of four cysteines to one zinc atom (see Fig. 1). Site-directed mutagenesis demonstrated that seven of eight cysteines are essential for receptor function.[35] The major grove of the DNA double helix has been shown to be a contact area.[36] In particular, the region spanning the carboxy terminal of the first zinc finger, the P box, is believed to be involved in the specificity of the binding to DNA.[37] The second carboxy terminal zinc finger is also required for DNA binding.[38] Five amino acids at the amino terminal base of the second zinc finger comprise the D-box, which is involved in the homodimerization of the GR by interacting with the equivalent part of the other DBD in a GR homodimer.[37,39] The amino acids responsible for the nuclear localization, the nuclear localization signal (NLS), are adjacent to the second zinc finger (see Fig. 1).[40] The carboxy terminal portion of the receptor includes the specific sequences for hormonal ligand binding, heat shock protein 90 (hsp90) binding, nuclear translocation, dimerization, and transactivation.

The covalent affinity labeling of the GR by steroids led to the identification of the contact amino acid residues

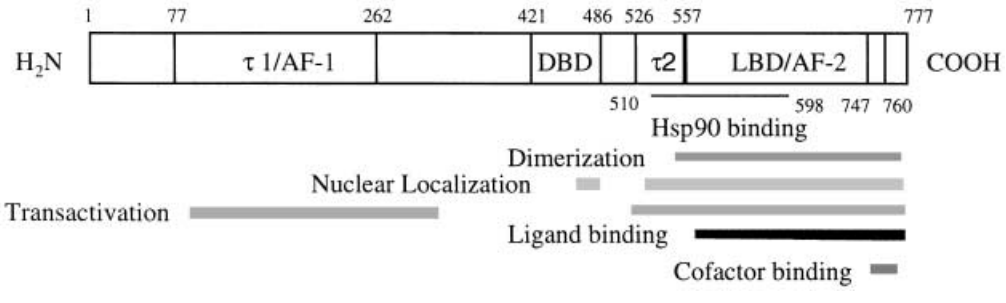

Fig. 1. Primary structure of the glucocorticoid receptor. *DBD*, DNA-binding domain; *LBD*, ligand-binding domain

Met622, Cys656, and Cys754 of the rat GR.[41] These amino acids are located in the hydrophobic segments within the LBD, indicating that ligand binds to the hydrophobic pocket-like structure.[41] Crystallographic analysis of the LBD confirmed this hypothesis in, for example, the ER.[42–45] The regions for hsp90 binding and the second NLS overlap the LBD.[46] The C-terminal transcriptional activation domain is hormone dependent and is called AF-2. The very end of AF-2, sometimes called the AF2-core, serves as a direct molecular switch that recruits coactivator proteins and activates the transcription of target genes when flipped into the active conformation by hormone binding.[47] The nuclear receptors thus contain distinct domains governing multiple functions, and their integration within the receptor may result in exquisite specificity of the hormonal response.

Mechanism of GR-dependent transcriptional regulation

Glucocorticoids, as lipophilic substances, are believed to cross the cell membrane readily. On a binding hormone, the GR dissociates hsp90 and translocates to the nucleus (Fig. 2).[48,49] The nuclear import of the GR is therefore one of the key control points in the regulation of glucocorticoid hormone action. In general, protein transport from the cytoplasm to the nucleus involves NLS, i.e., short peptide sequences that are necessary and sufficient for the nuclear localization of their respective proteins.[49,50] One of the best-characterized NLS motifs is that of simian virus 40 large tumor antigen (SV40 T-ag).[50] The nuclear import of the GR is mediated by NL1, a stretch of basic amino acids at the immediate C-terminal end of the receptor DBD,

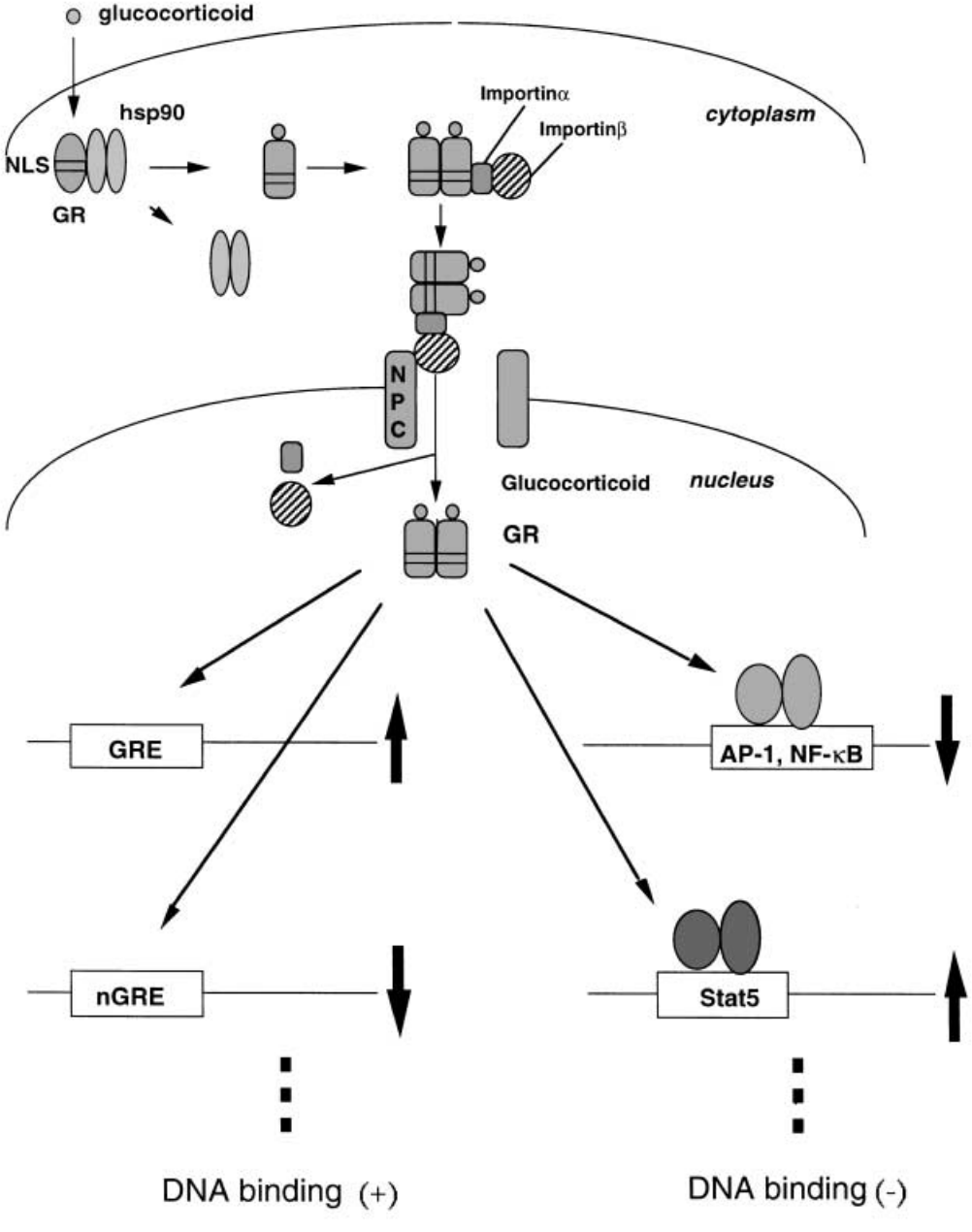

Fig. 2. The putative mechanisms of glucocorticoid receptor (*GR*)-mediated transcriptional regulation. The GR, on binding ligand, regulates gene expression in a variety of fashions. Interaction with glucocorticoid response element (*GRE*) and negative GRE (*nGRE*) requires DNA binding of the GR. In contrast, interaction with AP-1, nuclear factor (NF)-κB, and STAT5 does not require DNA binding of the GR, although the requirement of a DBD is suggested in some cases. *NLS*, nuclear localization signal; *NPC*, nuclear pore complex; *hsp90*, heat shock protein 90

and a second significantly less characterized NLS in the ligand binding domain, NL2.[40] Whereas the NLS of SV40 T-ag consists of a short domain of basic amino acids, the NL1 of the GR is bipartite, and confers constitutive nuclear localization of the receptor (see Fig. 1).[40] In contrast, NL2 acts as a dominant negative NLS in the absence of ligands.[40]

It is believed that the GR shuttles between the cytoplasm and the nucleus, and subcellular localization of the GR is determined by an equilibrium of nuclear import and export. The GR translocates to the nucleus in a ligand- and energy-dependent manner, and nuclear export of the GR also requires adenosine triphosphate (ATP).[51–60] Within the nucleus, the hormone-bound GR binds to the palindromic DNA sequences, called glucocorticoid response elements (GREs), exclusively as a homodimer (Fig. 2).[10,11,61] After binding to DNA, the GR is considered to communicate with basal transcription machinery, interacting with or without other transcription factors and coactivators, then regulating the target gene (Fig. 3).[10–12,62–64] A series of searching proteins interacting with the LBD converge on a family of related proteins that are collectively termed the p160 coactivators. They are represented by SRC-1/NCoA-1, TIF2/GRIP1/NCoA-2, and pCIP/ACTR/AIB1.[65–69]

As well as sequence homology, p160 proteins share an ability to stimulate ligand-dependent transactivation by a rather large number of the nuclear receptors. A distinct structural feature of the p160 coactivators is the presence of multiple LXXLL signature motifs (also called LXDs, NR boxes, or NIDs), which comprise determinants for direct interactions with the nuclear receptor LBD.[68] Although the amino acid context surrounding the LXXLL motif appears to influence the selectivity of the interaction, it is unclear at this point what, if anything, influences the specificity of binding between the nuclear receptor and the p160 proteins. Recent studies of the crystal structures of the LBD have established that upon ligand binding, the α-helix containing the AF2-core (helix 12) undergoes a major reorientation in the context of the overall LBD structure, forming part of a charged clamp that accommodates p160 coactivators within a hydrophobic cleft of the LBD; this occurs through direct contact with the LXXLL.[68] The estrogen antagonists tamoxifen and raloxifen appear to alter the position of the AF-2 core so that helix 12 itself occupies the hydrophobic cleft in the LBD, thereby precluding coactivator binding.[42,45]

Insight into a possible mechanism of p160 coactivation came with the finding that SRC-1 is capable of interacting with the C-terminus of CBP/p300, and together they can coactivate transcription synergistically.[69] In addition, CBP/p300 itself interacts with the nuclear receptors in a ligand-dependent manner, again through the AF-2 domain.[70,71] CBP/p300 and p160 coactivators both possess intrinsic histone acetylase (HAT) activity and therefore may be acting in concert to remodel chromatin.[69–72] Moreover, p/CAF, a mammalian homologue of the prototypical yeast HAT, GCN5, is part of a 20 or so subunit complex containing trans-active functions (TAFs) and TAF-like proteins,[72,73] and interacts with both CBP and some p160 coactivators, as well as directly with the nuclear receptors.[73,74] One can thus

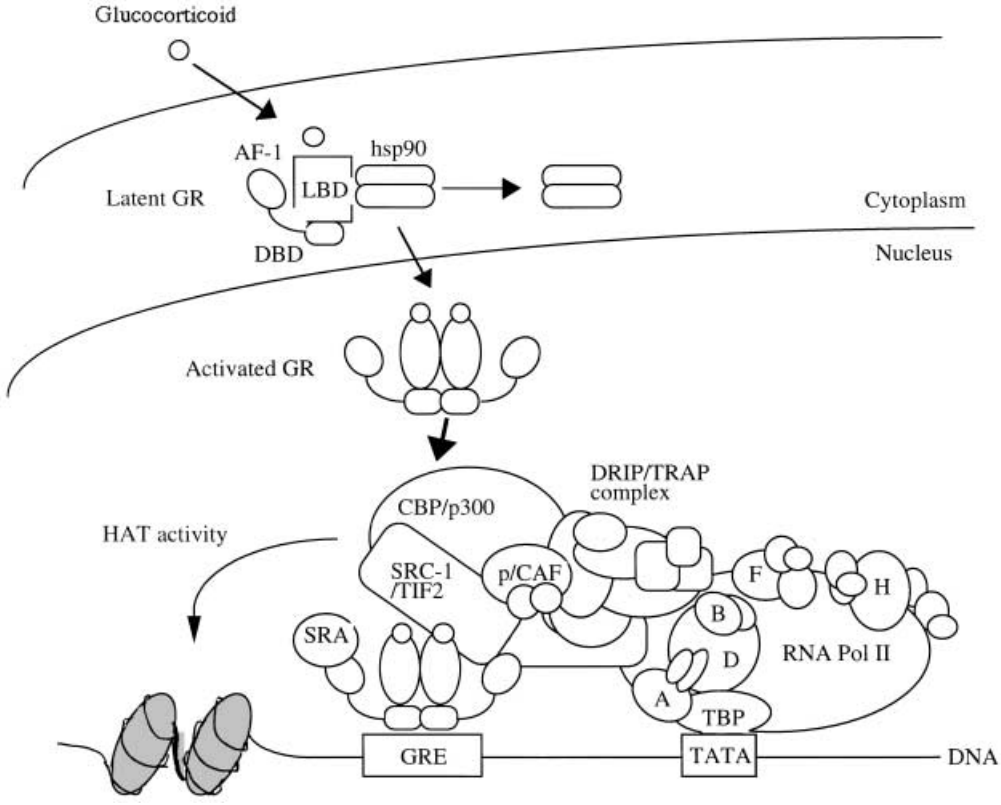

Fig. 3. Chromatin dynamics and the GR. The GR exists as part of a large protein complex which associates with, and modulates, chromatin. CBP, CREB binding protein; *DRIP*, vitamin D receptor interacting protein; *HAT*, hystone acetyltransferase; *p/CAF*, p300/CBP and the associated factor; *RNA* Pol II, RNA polymerase II; *SRA*, steroid receptor RNA activator; *SRC-1*, steroid receptor coactivator-1; TBP, TATA binding protein; *TIF2*, transcription intermediary factor 2; *TRAP*, TR-associated protein; *A*, *B*, TFIIA; *D*, TFIIA; *F*, TFIIA; *H*, TFIIA; tumor-associated transplantation antigen

imagine a growing HAT-containing, chromatin-remodeling complex comprised of CBP/p300, p160, and p/CAF recruited to the nuclear receptors for hormone binding (see Fig. 3). On the other hand, corepressors have been shown to deacetylate histones in combination with other nuclear proteins such as Sim3. Kang et al.[75] reported that in the case of the GR, hsp90 behaves as a corepressor in the nucleus.

In addition to coactivators and corepressors, another class of cofactor for the nuclear receptors, termed DRIP/TRAP, has been isolated.[76–79] Moreover, other chromatin remodeling machineries such as ATP-dependent chromatin remodeling complexes have been found; BAF, for example, is a mammalian homologue of yeast SWI/SNF.[80–82] Together, the fields of transcriptional regulation and chromatin structure and function have now been merged, and the regulation of gene expression by nuclear receptors is being described in a chromatin context. The disruption of interdomain and coactivator interactions was clinically implicated in the pathophysiology of a patient with oligospermic infertility associated with an androgen receptor mutation.[83]

In addition to ligand-dependent activation, extracellular signaling molecules such as peptide hormones, growth factors, and cytokines communicate with their intracellular targets through surface receptors. They activate signal transduction pathways that finally lead to the regulation of gene expression mediated by transcription factors such as c-Fos, c-Jun, cAMP-responsive element-binding protein, and others. The mechanism usually involves phosphorylation of those transcription factors by kinases that are activated as a result of the ligand–receptor interaction at the cell surface. The nuclear receptors are also indicated as being targets of some, but not all, kinases involved in signal transduction, and the phosphorylation of nuclear receptors provides an important mechanism for cross talk between signaling pathways.[84,85] Multiple kinase pathways have been implicated in the modulation of nuclear receptor-mediated gene regulation: cAMP-dependent protein kinase, casein kinase, glycogen synthase kinase (GSK), c-Jun kinase, cyclin-dependent kinases (CDKs), and mitogen-activated protein kinases (MAPKs).[84,85] All aspects of receptor function can be regulated by kinases, including DNA binding and dimerization, transcriptional activity, interaction with cofactors, and ligand binding. In the case of the GR, Ser246,[86] Ser224 and 232,[86] and Thr171,[31] all of which are located in the AF-1 region, are indicated as being targets of MAPK, CDKs, and GSK-3, respectively. In a physiological and/or pharmacological context, it should be noted that many effects of glucocorticoids are achieved not only by activation, but also by the inhibition of target gene expression (see Fig. 2).[7] This is particularly true for the antiinflammatory/immunosuppressive effects of glucocorticoids that involve the negative transcriptional regulation of proinflammatory genes.[87] This mode of regulation is distinct from the positive regulation described previously, and does not necessarily involve the interaction of the GR with GRE, but is achieved by the interaction between the GR and so-called negative GRE (nGRE) (see Fig. 2).[88] On the other hand, the expression of many proinflammatory genes is positively regulated by a certain class of transcription factors, for example, AP-1 and nuclear factor (NF)-κB.[87] The negative regulation of these genes by the GR is sometimes referred to as "cross talk" between the GR and these transcription factors, or transrepression (see Fig. 2).[89] Numerous molecular mechanisms have already been presented to account for such mutually exclusive interactions between transcription factors; e.g., direct protein–protein interaction, the squelching of coactivators, and inhibition of the catalytic activity of enzymes that modulate the transcription factors.[90–97] Moreover, it has recently been reported that the GRα could heterodimerize with other members of the nuclear receptor superfamily, including the GRβ,[17] MR,[98] and AR.[99] We have recently demonstrated that the GR function is regulated in a redox state-dependent manner.[100–103]

In conclusion, the GR, after activation by a ligand, elicits pleiotropic and conditional regulation of gene expression, which may allow the fine tuning of cellular metabolic processes and stress responses.

Future perspectives

Synthetic glucocorticoids have been shown to be a powerful tool for the treatment of a number of human diseases. It has been a long-standing goal of pharmacologic research to develop a glucocorticoid ligand that dissociates between transrepression and transactivation, since many of the side effects of conventional glucocorticoids can be attributed to transactivation.[104–107] A novel ligand may be developed by using a molecular pharmacologic technique based on knowledge gained from the structural analysis of the LBD. Members of the nuclear receptor superfamily for which the crystal structures have been elucidated have an LBD consisting of 12α-helices.[108,109] In the ER, there are already prototypical compounds called selective ER modulators.[110] In any case, the crystallography of GR LBD will make it theoretically possible to design a novel GR modulator. On the other hand, we have recently shown that a bile acid ursodeoxycholic acid (UDCA) could dissociate the transactivation and transrepression of NF-κB.[111] However, UDCA does not bind the GR, but mimics some of the GR functions, including NF-κB repression. It may thus be possible to speculate that a selective GR modulator does not necessarily contain a glucocorticoid structure.

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