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Human Glucocorticoid Receptor (GR) Isoform β : Recent Understanding of its Potential Implications in Physiology and Pathophysiology

Tomoshige Kino^{1,*}, Yan A. Su², and George P. Chrousos³

¹Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA

²GenProMarkers, Inc., Rockville, Maryland 20852, USA

³First Department of Pediatrics, Athens University, Athens 11527, Greece

Abstract

The human glucocorticoid receptor (GR) gene expresses two splicing isoforms α and β through alternative use of specific exons 9 α and 9 β . In contrast to the classic receptor GR α , which mediates most of the known actions of glucocorticoids, the functions of GR β have been largely unexplored. Owing to newly developed methods, such as microarrays and the jellyfish fluorescence proteins, we and others have recently revealed novel functions of GR β . Indeed, this enigmatic GR isoform influences positively and negatively the transcriptional activity of large subsets of genes, most of which are not responsive to glucocorticoids, in addition to its well-known dominant negative effect against GR α -mediated transcriptional activity. A recent report suggested that the “ligand-binding domain” of GR β is active, forming a functional ligand-binding pocket, associated with the synthetic compound RU 486. In this review, we discuss the functions of GR β , its mechanisms of action, and its pathologic implications.

Keywords

cytoplasmic to nuclear translocation; glucocorticoid receptor; ligand-binding pocket; microarray; splicing isoform; zebrafish

Introduction

Glucocorticoids, the end-products of the hypothalamic-pituitary adrenal axis, are steroid hormones crucial for the regulation of basal and stress-related homeostasis [1,2]. Glucocorticoids are also essential for the proper functioning of virtually all organs and tissues of the organism, including the central nervous (CNS) and cardiovascular systems, metabolic organs, such as the liver and adipose tissue, as well as the immune/inflammatory response [3,4]. In addition, glucocorticoids at “pharmacologic” or “stress-related” doses are irreplaceable therapeutic means for many allergic, inflammatory, autoimmune, and lymphoproliferative diseases [4].

*Address correspondence and requests for reprints to: Tomoshige Kino, M.D., Ph.D., Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bldg. 10, Clinical Research Center, Rm. 1E-3140, 10 Center Drive MSC 1109, Bethesda, MD 20892-1109, USA, Phone: 301-496-6417, Fax: 301-402-0884, kinot@mail.nih.gov.

The actions of glucocorticoids are mediated by a ubiquitous intracellular receptor protein, the glucocorticoid receptor (GR), which functions as a hormone-activated transcription factor of glucocorticoid target genes [5,⁶]. The human GR gene located in chromosome 5 and encodes two splicing variants GR α and GR β from alternative use of a different terminal exon 9 α and 9 β [5,⁷]. GR α is the classic receptor, binding to glucocorticoids and mediating most of the known glucocorticoid actions [5]. In contrast, GR β does not bind glucocorticoids but functions as a dominant negative inhibitor of GR α -induced transactivation of GRE-containing, glucocorticoid-responsive promoters; its physiologic/pathologic roles have not been well elucidated as yet [8,⁹].

Using the microarray technique, which enabled us to evaluate gene expression *en masse*, we and others recently found that the GR β isoform has intrinsic, GR α -independent transcriptional activity, in addition to its well-known dominant negative effect on GR α [10,¹¹]. In this review article, we will summarize known GR β activities and discuss newly identified actions of this GR isoform.

The human GR gene, splicing variants GR α and GR β , and their multiple translational isoforms

The GR, also called as nuclear receptor superfamily 3, group C, member 1 (NR3C1), belongs to the steroid/sterol/thyroid/retinoid/orphan nuclear receptor superfamily, which consists of over 130 members preserved from the early metazoans to humans [5,¹²]. The human GR gene, located in the short arm of chromosome 5 (5q31.3), consists of 9 exons, and its expression is regulated by at least three different promoters (A, B and C) [7,¹³], with promoter A alternatively used with three unique promoter fragments 1A1, 1A2 and 1A3 [13]. Thus the GR gene can produce five different transcripts from different promoters that encode the same GR proteins. In addition to alternative transcripts using the 5' different promoters, the GR gene generates two 3' splicing variant transcripts with alternative use of exon 9 α and/or 9 β (Figure 1). Thus, the GR gene generates 10 different transcripts that encode two protein molecules GR α and GR β .

Recently, it became evident that the GR α variant mRNA is translated from at least 8 initiation sites into multiple GR α isoforms termed A through D (A, B, C1–C3 and D1–D3), producing different amino terminal isoforms with distinct specific transcriptional activities on glucocorticoid-responsive genes [14] (Figure 1). These GR molecules are also differentially expressed in several different cell lines and tissues [14]. Given that GR α and GR β share a common mRNA domain that contains the same translation initiation sites [15], it appears that the GR β variant mRNA is also translated through the same initiation sites to a similar host of 8 β isoforms [5] (Figure 1).

The classic receptor GR α

GR α , the classic glucocorticoid receptor is ubiquitously expressed and mediates most of the known actions of glucocorticoids [3,⁵]. The human GR α consists of 777 amino acids and has 3 major distinct functional domains, the N-terminal or immunogenic domain (NTD), the DNA-binding domain (DBD) and the ligand-binding domain (LBD) [6] (Figure 1). The LBD of GR α consists of 12 α -helices and 4 β -sheets, among which helices 3, 4, 11 and 12 form the ligand-binding pocket for binding to glucocorticoids [16–18] (Figure 2). GR α is located primarily in the cytoplasm in the absence of glucocorticoid ligand, as part of hetero-oligomeric complexes containing heat shock proteins (HSPs) 90, 70, 50, 20 and, possibly, other proteins as well [5,⁶] (Figure 3). After binding to its agonist ligand, GR α undergoes conformational changes, dissociates from the heat shock proteins, homo-dimerizes, and translocates as a monomer or dimer into the nucleus through the nuclear pore, via an active ATP-dependent

process mediated by its nuclear localization signals (NL)-1 and -2 [12,¹⁹]. NL-1 is located in the junction of DBD and the hinge region, while NL-2 spans the entire LBD [19] (Figure 1).

Inside the nucleus, the ligand-activated GR α directly interacts as a dimer with specific DNA sequences, the glucocorticoid response elements (GREs), in the promoter regions of target genes, or as a monomer or dimer with other transcription factors via protein-protein interactions, indirectly influencing the activity of the latter on their own target genes [5,¹²] (Figure 3). GR contains two transactivation domains, activation function (AF)-1 and -2, located at its NTD and LBD, respectively, through which the GR interacts with many proteins and protein complexes, such as the nuclear receptor coactivator [p160, p300/CREB-binding protein (CBP) and p300/CBP-associated factor (p/CAF)] complexes and the SWI/SNF and vitamin D receptor-interacting protein/thyroid hormone receptor-associated protein (DRIP/TRAP) chromatin-remodeling complexes, eventually influencing the activity of RNA polymerase II and its ancillary factors, altering the transcription rates of glucocorticoid-responsive genes [5,^{6,20}] (Figure 1).

GR also interacts with the nuclear receptor corepressor (NCoR) and its homolog silencing mediator of retinoic acid and thyroid hormone receptor (SMRT), which are macromolecular docking platforms for nuclear receptors and many transcription factors, repressing the transcriptional activity of the GR by attracting histone deacetylase/Sin3 complexes [20]. The p160 type coactivators and the NCoR/SMRT type corepressors establish equilibrium in their interaction with the GR to respectively facilitate or block its transcriptional activity [21]. Accumulation of coactivators and corepressors on the promoter-bound GR is dependent on the kind of ligands bound to the GR: agonist glucocorticoids attract the coactivator complexes to the promoter-bound GR, while antagonists, like RU 486, accumulate the corepressor complexes [22] (Figure 2).

In addition to transactivation of the glucocorticoid-responsive genes explained above, GR α modulates other signal transduction cascades through mutual protein-protein interactions with specific transcription factors, by influencing their ability to stimulate or inhibit the transcription rates of their respective target genes (Figure 3). This activity may be more important than the GRE-mediated one, granted that mice harboring a mutant GR α , which is active in terms of protein-protein interactions but inactive in terms of transactivation via DNA GREs, survive and procreate, in contrast to mice with a deletion of the entire GR gene that die immediately after birth from severe respiratory distress syndrome [23,²⁴]. The former mouse model and additional *in vitro* results indicate that GR interacts with and influences other transcription factors primarily as a monomer [23,²⁵].

The protein-protein interactions of GR α with other transcription factors may take place on promoters that do not contain GREs (tethering mechanism), as well as on promoters that have both GRE(s) and responsive element(s) of transcription factors that interact with GR α ("composite promoters") [26]. Suppression of transactivation of other transcription factors through protein-protein interactions may be particularly important in the suppression of immune function and inflammation by glucocorticoids [23,²⁵]. A substantial part of the effects of glucocorticoids on the immune system may be explained by the interaction between GR α with nuclear factor- κ B (NF- κ B), activator protein-1 (AP-1) and probably the signal transducers and activators of transcription (STATs) [27–³⁰].

In addition to co-regulators and other transcription factors that modulate GR-induced transcriptional activity, several distinct signaling pathways regulate the transcriptional activity of the GR via post-translational modifications of the receptor protein [5]. These include methylation, acetylation, nitrosylation, sumoylation and ubiquitination, as well as phosphorylation, which has been studied best. Indeed, several kinases, such as the cell-cycle-

related kinases, mitogen-activated kinases and the glycogen synthase kinases, phosphorylate specific serine or threonine residues of the GR. Interestingly, the majority of these residues are located in the AF-1 domain of the human GR NTD, thus phosphorylation of some or all of them modulates GR-induced transcriptional activity through alteration of co-regulator attraction to the promoter region of glucocorticoid-responsive genes, possibly by changing their affinity to the AF-1 domain of GR [31].

The splicing variant GR β isoform

Similarly to the classic human GR α , the original human GR β isoform is also ubiquitously expressed in most tissues. This isoform was identified in both the zebrafish and humans, but not in mice [15,^{32,33}]. The human (h) GR β contains 742 amino acids and shares the first 727 amino acids from the N-terminus with hGR α [6,¹⁵] (Figure 1). hGR β encodes an additional 15 nonhomologous amino acids in the C-terminus, while hGR α possesses an additional 50 amino acids forming a 777 amino acid protein [6,¹⁵] (Figure 1). Therefore, hGR β shares the same NTD and DBD with hGR α , but has a unique “LBD”. Since the divergence point (amino acid 727) is located at the C-terminal end of the helix 10 in the hGR α LBD, the hGR β “LBD” does not have the helices 11 and 12 of the hGR α . As these helices are important for forming the ligand-binding pocket and for the creation of the AF-2 surface upon ligand binding [16] (Figure 2), GR β cannot form an active ligand-binding pocket, does not bind glucocorticoids, and thus, does not directly regulate GRE-containing, glucocorticoid-responsive gene promoters. In the absence of the hGR β “LBD”, the truncated hGR consisting of NTD and DBD is transcriptionally active on GRE-containing promoters [34], thus the hGR β “LBD” somehow attenuates the transcriptional activity of the other subdomains of the molecule on GRE-driven promoters. Inside the cells, hGR β can localize both in the cytoplasm and nucleus [9,³⁵].

Similarly to the human GR gene, the zebrafish (z) GR gene consists of 9 exons and produces the zGR α and zGR β proteins, which respectively contain 746 and 737 amino acids [32] (Figure 4). zGR α and zGR β share the N-terminal 697 amino acids, while they have specific C-terminal portions, which contain 47 and 40 amino acids, respectively. In contrast to hGR α and hGR β , which are produced through alternative use of specific exon 9 α and 9 β , zGR α and zGR β are formed as a result of intron retention [32]. zGR α and zGR β use exon 1 to exon 8 for their common N-terminal 697 amino acids. zGR α uses exon 9 for its specific C-terminal portion, while zGR β continuously employs the rest of exon 8 and uses a stop codon located at 3' portion of this exon to express its specific C-terminal peptide [32] (Figure 4). Protein alignment comparison of hGR β and zGR β indicated that these two molecules employ exactly the same divergence point, while their β isoform-specific C-terminal peptides show little sequence homology [32]. These pieces of molecular information indicate that hGR β and zGR β evolved independently. Nevertheless, zGR β demonstrated the same functional properties as those of the hGR β , such as inability to bind glucocorticoids, a dominant negative activity on zGR α transcriptional activity on GRE-drive promoters, and strikingly similar tissue distribution as hGR β [32]. Thus, hGR β and zGR β are produced through convergent evolution, most likely developed through strong requirement of this type of GR isoform in a physiologic situation.

The presence of nonligand-binding C-terminal variants is not unique to the GR. Similarly to the human and zebrafish GR, several other human steroid and nuclear receptors, such as the estrogen receptor β (ER β), thyroid hormone receptor α (TR α), vitamin D receptor, constitutive androstane receptor (CAR), dosage-sensitive sex reversal-1 (DAX-1), nuclear receptor related2 (Nurr2), neuron-derived orphan receptor-2 (NOR-2), peroxisome proliferator-activated receptor α (PPAR α), and PPAR γ , also have C-terminally truncated receptor isoforms defective in binding to cognate ligands with dominant negative activity on their corresponding classic receptors [36–45]. This suggests that evolution has allowed the development and retention of such alternative nuclear receptors, probably because they play useful biologic roles.

The dominant-negative effect of GR β on GR α -induced transcriptional activity: Physiologic and pathologic implications

In contrast to GR α that has numerous and diverse actions [3], the functions of GR β had not been revealed until we reported its dominant negative effect on GR α -induced transcriptional activity almost a decade after the original identification of this receptor isoform [8]. The dominant negative activity of GR β was first demonstrated in transient transfection-based reporter assays using GRE-driven reporter genes, but was subsequently confirmed on endogenous, glucocorticoid-responsive genes, such as the mitogen-activated protein kinase phosphatase-1 (MPK-1), myocilin and fibronectin [46, 47]. Further, GR β was shown to attenuate glucocorticoid-induced repression of the tumor necrosis (TNF) α and interleukin (IL)-6 genes [46]. We also confirmed this negative effect of GR β on GR α -mediated transrepression using microarray analyses [10]. Several mechanisms explaining this GR β function have been reported, including (1) competition for GRE binding through their shared DBD, (2) heterodimerization with GR α and (3) coactivator sequestration through the preserved AF-1 domain [8, 34, 48]. All these different mechanisms of action appear to be functional, depending on the promoters and tissues affected by this GR isoform.

Several clinically oriented investigations suggest that GR β is responsible for the development of tissue-specific insensitivity to glucocorticoids in various disorders, most of them associated with dysregulation of immune function. They include glucocorticoid-resistant asthma, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), ankylosing spondylitis, chronic lymphocytic leukemia and nasal polyps [49–55]. In these studies, various immune cells expressed elevated levels of GR β , which correlated with reduced sensitivity to glucocorticoids. Elevated levels of pro-inflammatory cytokines, such as IL-1, -2, -4, -7, -8 and -18, TNF α , and interferons α and γ , might have been responsible for increased GR β expression in cells from patients with these pathologic conditions, as these cytokines experimentally stimulated the expression of GR β in lymphocytes, neutrophils or airway smooth muscle cells [56–61]. Further, presence of a single nucleoside polymorphism in the 3' untranslated region of the hGR β mRNA (rs6198G allele), which increases the stability of the mRNA, and thus, causes elevated expression of GR β protein, was associated with increased incidence of RA, SLE, high blood pressure, ischemic heart diseases and nasal carriage of *Staphylococcus aureus* [50, 62–64], possibly through inhibition of glucocorticoid actions by increased concentrations of GR β . These pieces of clinical evidence further support that GR β has dominant negative activity on GR α -induced transcription inside the human body, functioning as a negative regulator of glucocorticoid actions in local tissues.

GR β has intrinsic, GR α -independent transcriptional activity

We and others recently performed transcriptome analyses using microarray techniques in cultured cells overexpressing GR β , and found that these cells had a distinct mRNA expression profile compared to cells not overexpressing GR β and those expressing GR α and treated with glucocorticoids [10, 11]. In a subsequent real-time PCR analysis, we also confirmed that GR β regulates mRNA expression positively and negatively in a gene-specific fashion [10]. These results indicate that GR β has intrinsic transcriptional activities independent of the activity of its isoform GR α . We have compared the microarray results obtained by us and those of others [10, 11], and found that the 2 studies share 78 genes modulated by overexpression of GR β (Table 1). Specifically, 29 out of 78 genes were both down-regulated by GR β overexpression, while only 8 were up-regulated. Interestingly, 41 genes showed opposite response to GR β between the two studies, suggesting that GR β modulates mRNA expression of some of its responsive genes in a cell-, and possibly, a cell culture condition-specific fashion.

Apparently, this intrinsic transcriptional activity of GR β is not mediated by binding of the isoform to classic GREs, as GR β does not influence the transcriptional activity of classic GREs-driven promoters, while the promoter region of the genes which we identified to be regulated by GR β , do not contain GRE sequences [10]. Rather, GR β directly modulates the transcriptional activity of its responsive genes, which are distinct from those responsive to glucocorticoids, possibly by altering the activity of transcriptional intermediate molecules or other transcription factors through physical protein-protein interactions. Indeed, we previously demonstrated that the AF-1 of the GR β , which presumably keeps the same protein structure and function as that of the GR α , is transcriptionally active, contributing to its dominant negative activity against GR α -induced transactivation [34]. This transactivation domain of GR α interacts with numerous cofactor molecules, including CBP/p300 and p160-type histone acetyltransferase coactivators, components of the SWI/SNF chromatin modulators, DRIP150 of the DRIP/TRAP complex and the steroid receptor RNA coactivator (SRA) [65–70]. Thus, it is possible that GR β alters the transcriptional activity of its responsive genes by lodging into the transcriptional complexes formed on their promoter region through its AF-1 (Figure 5). This mechanistic hypothesis is further supported by the recent results from other groups, in which GR β was shown to repress the transcriptional activity of AP-1 and NF κ B, possibly through protein-protein interactions similar to those between GR α and these transcription factors [71].

GR β was also reported to suppress the transcriptional activity of the GATA3 transcription factor on its responsive IL-5 and -13 promoters by attracting histone deacetylases [72]. Alternatively, GR β might bind DNA sequences unique to this isoform through its DBD, regulating transcription through hypothetical “GR β REs” (Figure 5). Since the subdomains of steroid hormone receptors influence each other’s activity [73,74], the unique GR β “LBD” might alter the binding specificity of its DBD to DNA and allow it to recognize a set of DNA sequences specific to GR β and distinct from those of GR α .

The importance and exact roles of this intrinsic transcriptional activity of the GR β isoform in physiology and pathophysiology have not been elucidated as yet. We have performed a pathway analysis of our microarray results to define the biologic pathways where GR β might play consistent roles [10], and found that this GR isoform may be involved in regulation of 43 distinct pathways recorded in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Table 2). Among the pathways we found in this analysis, GR β might strongly influence several cellular functions, such as cell communications (#13), focal adhesion (#26), ECM-receptor interaction (#27), expression of cell adhesion molecules (#28) and regulation of actin cytoskeleton (#34), as well as metabolism of some amino acids and other bioactive molecules. Interestingly, GR β might also play a role in the development/activity/apoptosis of cancer cells, as it also regulates mRNA expression of genes important for colorectal (#40), renal cell (#41), prostate (#42), and small cell lung cancer (#43) and apoptosis (#23). To further verify the biologic pathways, in which GR β plays important roles, development of the mice conditionally overexpressing human GR β would be very helpful.

Issues on “ligand” and subcellular localization of GR β

A previous publication demonstrated that only RU 486 among 57 native and synthetic steroids tested bound GR β weakly at the “ligand-binding” pocket of the GR β and slowly (over 6 hours for completion) induced its nuclear translocation [11]. The results were supported by a nuclear translocation study using fluorescent protein-fused GR β by scoring cellular localization of this fusion protein in different cells, by a whole-cell ligand-binding assay followed by the crude fractionation of radiolabeled ligand-associated receptors with a Sephadex column and by computer-based modeling of the GR β “ligand-binding domain” associated with several steroids [11]. This report also demonstrated that RU 486 modulated GR β -mediated

transcriptional activity in microarray analysis [11]. Although the hypothesis presented in this publication is interesting, there are several points to be resolved. Yet undiscovered endogenous steroids or other related compounds with structures similar to that of RU 486 would be expected to be the endogenous ligands of GR β . Crystallographic structural analysis of the GR β “LBD” might help identifying a “ligand-binding pocket” in the GR β “LBD” and hence its binding to RU 486. The cytoplasmic to nuclear translocation of GR β demonstrated by the previous report was quite slow compared to that of GR α : in the former, the receptor took 6 hours to complete its translocation, while in the latter it did this within minutes [11]. GR β and GR α share NL-1, which mediates the rapid nuclear translocation of GR α , while GR β does not appear to have NL-2, which is dependent on the entire LBD of GR α , and causes slower nuclear translocation of the receptor [19]. Thus, presence of yet unknown regulators specific to GR β might be involved in the nuclear translocation of this isoform.

We independently performed several experiments addressing the potential activation of GR β by RU 486, its subcellular localization and cytoplasmic to nuclear translocation. In contrast to the previously reported findings [11], the green fluorescent protein-fused GR β was mainly localized in the nucleus in HeLa cells stably expressing this fusion protein, while it was heterogeneously distributed both in the cytoplasm and the nucleus in HCT116 cells that expressed the GR β fusion protein transiently: some cells mainly expressed GR β in the nucleus, while others had it in the cytoplasm [10]. Addition of RU 486 did not stimulate the transcriptional activity of glucocorticoid-responsive and GRE-containing mouse mammary tumor virus promoter in transiently GR β -expressing HCT116 cells, and did not induce cytoplasmic to nuclear translocation of this isoform [10]. The inconsistency of our results with the previous report may have been caused by use of different experimental systems, such as cell lines and plasmids. This discrepancy suggests that the mechanisms of the regulatory actions of GR β on the transcription of responsive genes inside the cells are quite complex.

Summary

In 1995, ten years after the original identification of the human GR β by R. Evans’ group [15], we reported that GR β had a dominant negative effect on GR α -induced transcriptional activity, an effect that was replicated a year later [8,³⁵]. After another decade, a new activity of GR β , namely an intrinsic, GR α -independent transcriptional activity, was discovered by employing microarray-based transcriptome analyses [10,¹¹]. In spite of a continuous effort spanning over 20 years, the molecular mechanisms of action and the roles of GR β in physiology are still largely unknown, in contrast to those of the classic, glucocorticoid action-mediating GR α . The β isoform cannot modulate the transcriptional activity of GRE-containing promoters in the absence of GR α , even though it shares a perfect DBD with GR α [10]. Lack of GR β in rodents stands against the elucidation of its *in vivo* activity [33]. We hope that physiologic and pathologic roles of GR β will be further clarified with future technical progress, such as development of mice conditionally expressing human GR β , sophisticated transcriptome/promoter/proteome analyses with array techniques, evaluation of GR β subcellular circulation/localization through fusion with fluorescent proteins and crystallography-based structural analyses.

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Figure 1. Genomic and complementary DNA and protein isoforms of the human GR and distribution of functional domains in its linearized molecule

The human GR gene consists of 9 exons. Exon 1 is an untranslated region, exon 2 codes for the N-terminal “immunogenic” domain, exons 3 and 4 for the DNA-binding domain, and exons 5–9 for the hinge region and the ligand-binding domain. The GR gene contains two terminal exons 9 (9 α and 9 β), which produce the classic GR α (GR α -A) and the GR β (GR β -A) through alternative splicing of these exons. C-terminal gray colored domains in GR α -A and GR β -A show their specific portions. GR α N-terminal translational isoforms expressed from a single GR α transcript are shown in the middle of the figure. Similar N-terminal translational isoforms may also be produced from the GR β -specific transcript using the same start sites (modified from [5]).

AF-1 and -2: activation function 1 and 2; DBD; DNA-binding domain; HD: hinge region; LBD: Ligand-binding domain; NL1 and 2: Nuclear translocation signal 1 and 2, NTD: N-terminal domain.

Dexamethasone-bound Form

RU 486-bound Form

Figure 2. The 3-dimensional structure of GR α associated with agonist dexamethasone (left) and antagonist RU 486 (right)

Results of the crystallographic analyses of the GR α associated with agonist dexamethasone (left) or with antagonist RU 486 (right) are shown [16,⁷⁵]. The LBD of GR α consists of 12 α -helices and 4 β -sheets, among which helices 3, 4, 11 and 12 form the ligand-binding pocket for binding to glucocorticoids. Helix 12 changes its localization dramatically upon binding to ligands, playing a critical role in the formation of a binding surface for the coactivator (LXXLL) motif. Image sources were downloaded from the RCSB Protein Data Bank (<http://www.rcsb.org>), while the images were created using the MacPyMOL software. Yellow bold arrow: ligand-binding pocket; white arrow: Helix 12; white arrowhead: the coactivator motif peptide fragment of the transcriptional intermediate factor 2.

Figure 3. Nucleocytoplasmic shuttling and transcriptional regulation of GR α

Upon ligand binding, the activated GR α dissociates from the heat shock proteins (HSPs) and translocates into the nucleus, where it binds as a homodimer to GREs in the promoter regions of target genes or interacts as a monomer with other transcription factors.

GR α : glucocorticoid receptor α ; GRE: glucocorticoid response element; HSPs: heat shock proteins; REs: response elements; RNPII: RNA polymerase II; TF: transcription factor.

Figure 4. Genomic and complementary DNA and protein isoforms of the zebrafish GR
 The zebrafish (z) GR gene consists of 9 exons. The zGR gene expresses zGRα and zGRβ splicing variants through intron retention [32]. C-terminal gray colored and shaded domains in zGRα and zGRβ show their specific portions. They are respectively encoded by exon 9 and the 3' portion of exon 8, which are also shown in the same labeling. DBD; DNA-binding domain; LBD: Ligand-binding domain; NTD: N-terminal domain; UTR: untranslated region.

Figure 5. Hypothetical models for GR β -mediated modulation of the transcriptional activity of its responsive genes

A: Through AF-1 located in the NTD, GR β may interact with numerous transcriptional cofactors and transcriptional factors, lodge into the transcription intermediate complex formed on the promoter region of GR β -responsive genes and modulate their transcriptional activity. GR β may attract histone deacetylases to the transcription intermediate complex formed on the promoter region of genes regulated by this GR isoform.

B: GR β might also bind to hypothetical specific response elements located in the promoter region of responsive genes, directly modulating their transcriptional activity. GR β : glucocorticoid receptor β ; HDACs: histone deacetylases; REs: response elements; RNPII: RNA polymerase II; TF: transcription factor.

Table 1

Seventy-eight genes regulated by GR β overexpression in HeLa and U-2 OS cells observed in 2 independent studies [10, 11]

No.	Gene ID	Gene Symbol	HeLa Cells*		U-2OS Cells**		Gene Name
			Change	p-Value	Change	p-Value	
1	4128	MAOA	-1.70	0.024	-1.80	0.000	Monoamine oxidase A
2	3431	SP110	-0.15	0.005	-1.04	0.007	SP110 nuclear body protein
3	7421	VDR	-0.25	0.041	-1.01	0.001	Vitamin D receptor
4	6653	SORL1	-0.37	0.027	-0.97	0.009	Sortilin-related receptor
5	4673	NAP1L1	-0.33	0.018	-0.96	0.006	Nucleosome assembly protein 1-like 1
6	596	BCL2	-0.30	0.006	-0.95	0.007	B-cell CLL/lymphoma 2
7	567	B2M	-0.43	0.039	-0.91	0.001	β 2-Microglobulin
8	902	CCNH	-0.28	0.015	-0.90	0.017	Cyclin H
9	23011	RAB21	-0.40	0.013	-0.86	0.015	RAB21, member RAS oncogene family
10	4697	NDUFA4	-0.33	0.017	-0.83	0.024	NADH dehydrogenase 1 a subcomplex, 4, 9kDa
11	2280	FKBP1A	-0.32	0.030	-0.81	0.008	FK506 binding protein 1A, 12kDa
12	79600	FLJ11217	-1.93	0.031	-0.79	0.001	Tectonic
13	5908	RAP1B	-0.25	0.006	-0.75	0.001	RAP1B, member of RAS oncogene family
14	481	ATP1B1	-0.49	0.017	-0.71	0.022	ATPase, Na ⁺ /K ⁺ transporting, β 1 polypeptide
15	2123	EV12A	-0.46	0.018	-0.70	0.000	Ecotropic viral integration site 2A
16	9111	NMI	-0.93	0.045	-0.69	0.008	N-myc interactor
17	648	BMI1	-0.58	0.017	-0.69	0.016	B lymphoma Mo-ML V insertion region
18	23429	RYBP	-0.37	0.000	-0.65	0.020	RING1 and YY1 binding protein
19	8411	EEA1	-0.36	0.007	-0.63	0.009	Early endosome antigen 1, 162kD
20	5480	PPIC	-0.43	0.040	-0.62	0.038	Peptidylprolyl isomerase C
21	4659	PPP1R12A	-0.89	0.019	-0.59	0.009	Protein phosphatase 1, regulatory subunit 12A
22	2958	GTF2A2	-0.69	0.015	-0.58	0.017	General transcription factor IIA, 2
23	1854	DUT	-0.44	0.001	-0.55	0.033	dUTP pyrophosphatase
24	4698	NDUFA5	-0.29	0.009	-0.55	0.016	NADH dehydrogenase 1 a subcomplex, 5, 13kDa
25	7322	UBE2D2	-0.19	0.000	-0.51	0.017	Ubiquitin-conjugating enzyme E2D 2
26	2184	FAH	-0.29	0.045	-0.48	0.007	Fumarylacetoacetate hydrolase
27	91137	LOC91137	-0.37	0.018	-0.46	0.019	Hypothetical protein BC017169
28	4637	MYL6	-0.88	0.014	-0.42	0.016	Myosin, light polypeptide 6

No.	Gene ID	Gene Symbol	HeLa Cells*		U-2OS Cells**		Gene Name
			Change	p-Value	Change	p-Value	
29	80011	NIP30	-0.47	0.014	-0.39	0.016	NEFA-interacting nuclear protein NIP30
1	3371	TNC	-4.62	0.017	0.80	0.006	Tenascin C (hexabrachion)
2	2048	EPHB2	-2.33	0.039	0.63	0.004	EPH receptor B2
3	1000	CDH2	-2.05	0.000	1.85	0.000	Cadherin 2, type 1, N-cadherin
4	9590	AKAP12	-1.28	0.025	1.58	0.000	A kinase (PRKA) anchor protein (gravin) 12
5	3688	ITGB1	-1.14	0.029	0.65	0.013	Integrin, β 1
6	10425	ARIH2	-1.04	0.001	0.66	0.003	Ariadne homolog 2 (Drosophila)
7	26018	LRIG1	-1.03	0.020	0.63	0.010	Leucine-rich repeats and immunoglobulin-like domains 1
8	3675	ITGA3	-0.76	0.024	0.46	0.021	Integrin, α 3
9	892	CCNC	-0.73	0.010	0.84	0.000	Cyclin C
10	3069	HDLBP	-0.72	0.005	0.87	0.000	High density lipoprotein binding protein
11	1284	COL4A2	-0.68	0.008	0.69	0.037	Collagen, type IV, α 2
12	21	ABCA3	-0.65	0.015	0.46	0.008	ATP-binding cassette, sub-family A, member 3
13	7204	TRIO	-0.60	0.032	0.52	0.006	Triple functional domain (PTPRF interacting)
14	1490	CTGF	-0.60	0.000	1.61	0.028	Connective tissue growth factor
15	7486	WRN	-0.60	0.014	0.40	0.038	Werner syndrome
16	51665	ASB1	-0.60	0.036	1.00	0.001	Ankyrin repeat and SOCS box-containing 1
17	6138	RPL15	-0.47	0.006	0.53	0.006	Ribosomal protein L15
18	10951	CBX1	-0.46	0.037	0.70	0.012	Chromobox homolog 1
19	55023	PHIP	-0.34	0.029	0.54	0.018	Pleckstrin homology domain interacting protein
20	1363	CPE	-0.33	0.042	1.06	0.025	Carboxypeptidase E
21	1841	DTYMK	-0.32	0.007	0.61	0.005	Deoxythymidylate kinase
22	6161	RPL32	-0.25	0.041	0.41	0.032	Ribosomal protein L32
23	6567	SLC16A2	-0.24	0.036	0.82	0.016	Solute carrier family 16, member 2
24	1947	EFNB1	-0.23	0.015	0.68	0.008	Ephrin-B1
25	8692	HYAL2	-0.16	0.024	1.07	0.000	Hyaluronoglucosaminidase 2
1	2729	GCLC	0.12	0.008	-0.77	0.004	Glutamate-cysteine ligase, catalytic subunit
2	5423	POLB	0.18	0.001	-0.44	0.021	Polymerase (DNA directed), b

No.	Gene ID	Gene Symbol	HeLa Cells*		U-2OS Cells**		Gene Name
			Change	p-Value	Change	p-Value	
3	6391	SDHC	0.22	0.039	-0.78	0.018	Succinate dehydrogenase complex, subunit C
4	3556	IL1RAP	0.23	0.013	-0.47	0.027	Interleukin 1 receptor accessory protein
5	821	CANX	0.32	0.035	-0.52	0.005	Calnexin
6	55052	MRPL20	0.36	0.011	-0.55	0.037	Mitochondrial ribosomal protein L20
7	483	ATP1B3	0.41	0.037	-0.53	0.002	ATPase, Na ⁺ /K ⁺ transporting, β 3 polypeptide
8	58488	PCTP	0.43	0.022	-0.72	0.011	Phosphatidylcholine transfer protein
9	949	SCARB1	0.43	0.005	-1.03	0.002	Scavenger receptor class B, member 1
10	10899	JTB	0.52	0.013	-0.38	0.011	Jumping translocation breakpoint
11	8520	HAT1	0.52	0.013	-0.59	0.029	Histone acetyltransferase 1
12	672	BRCA1	0.63	0.020	-0.36	0.025	Breast cancer 1, early onset
13	4666	NACA	0.67	0.021	-0.55	0.004	Nascent-polypeptide-associated complex a
14	2551	GABPA	0.82	0.011	-0.71	0.014	GA binding protein transcription factor, a
15	7003	TEAD1	1.13	0.029	-0.56	0.044	TEA domain family member 1
16	6590	SLPI	1.51	0.013	-0.77	0.030	Secretory leukocyte peptidase inhibitor
1	3913	LAMB2	0.20	0.048	0.51	0.025	Laminin, β 2
2	928	CD9	0.24	0.046	0.77	0.023	CD9 molecule
3	432	ASGR1	0.28	0.050	0.74	0.004	Asialoglycoprotein receptor 1
4	57799	RAB40C	0.38	0.011	0.35	0.007	RAB40C, member RAS oncogene family
5	7298	TYMS	0.41	0.012	1.20	0.001	Thymidylate synthetase
6	95	ACY1	0.57	0.019	0.59	0.002	Aminoacylase 1
7	4316	MMP7	1.41	0.000	1.67	0.032	Matrix metalloproteinase 7
8	5797	PTPRM	1.48	0.048	0.86	0.000	Protein tyrosine phosphatase, receptor type, M

* and **: Data were respectively from [10] and [11] (GEO: <http://www.ncbi.nlm.nih.gov/geo>, GEO Series accession number GSE5310).

Changes are shown in log2 values.

Numbers in red and green fonts indicate up-regulation and down-regulation by GR β overexpression, respectively.

Thirty-six genes regulated by GRβ overexpression in HeLa cells* are involved in 43 distinct biologic pathways in KEGG.

Table 2

No.	Gene Symbol	Pathways in KEGG	No.	Pathways in KEGG	Gene Symbol
1	ABCA3	hsa02010: ABC transporters – General	1	hsa00020: Citrate cycle (TCA cycle)	SDHC
2	ACY1	hsa00220: Urea cycle and metabolism of amino groups	2	hsa00190: Oxidative phosphorylation	NDUFA4
3	B2M	hsa04612: Antigen processing and presentation,			NDUFA5
4	BCL2	hsa01510: Neurodegenerative Diseases			SDHC
		hsa04210: Apoptosis	3	hsa00220: Urea cycle and metabolism of amino groups	ACY1
		hsa04510: Focal adhesion			MAOA
		hsa05030: Amyotrophic lateral sclerosis (ALS)	4	hsa00240: Pyrimidine metabolism	DUT
		hsa05060: Prion disease			TYMS
		hsa05210: Colorectal cancer			DTYMK
		hsa05215: Prostate cancer	5	hsa00251: Glutamate metabolism	GCLC
		hsa05222: Small cell lung cancer	6	hsa00260: Glycine, serine and threonine metabolism	MAOA
5	BRCA1	hsa04120: Ubiquitin mediated proteolysis	7	hsa00340: Histidine metabolism	MAOA
6	CANX	hsa04612: Antigen processing and presentation,	8	hsa00350: Tyrosine metabolism	FAH
7	CCNH	hsa04110: Cell cycle			MAOA
8	CD9	hsa04640: Hematopoietic cell lineage	9	hsa00360: Phenylalanine metabolism	MAOA

No.	Gene Symbol	Pathways in KEGG	No.	Pathways in KEGG	Gene Symbol
9	CDH2	hsa04514: Cell adhesion molecules (CAMs)	10	hsa00380: Tryptophan metabolism	MAOA
10	COL4A2	hsa01430: Cell Communication hsa04510: Focal adhesion hsa04512: ECM-receptor interaction hsa05222: Small cell lung cancer	11	hsa00480: Glutathione metabolism hsa00531: Glycosaminoglycan degradation hsa00643: Styrene degradation hsa00670: One carbon pool by folate	GCLC HYAL2 FAH TYMS
11	CPE	hsa04940: Type 1 diabetes mellitus	12	hsa01032: Glycan structures – degradation	HYAL2
12	DTYMK	hsa00240: Pyrimidine metabolism,	13	hsa01430: Cell Communication	COL4A2
13	DUT	hsa00240: Pyrimidine metabolism			LAMB2
14	EFNB1	hsa04360: Axon guidance			TNC
15	EPHB2	hsa04360: Axon guidance	14	hsa01510: Neurodegenerative Diseases	BCL2
16	FAH	hsa00350: Tyrosine metabolism hsa00643: Styrene degradation	15	hsa02010: ABC transporters – General	ABCA3
17	GCLC	hsa00251: Glutamate metabolism hsa00480: Glutathione metabolism	16	hsa03010: Ribosome	RPL32
18	GTF2A2	hsa03022: Basal transcription factors	17	hsa03022: Basal transcription factors	GTF2A2
19	HYAL2	hsa00531: Glycosaminoglycan degradation hsa01032: Glycan structures – degradation	18	hsa03030: DNA polymerase	POLB
20	IL1RAP	hsa04060: Cytokine-cytokine receptor interaction	19	hsa04010: MAPK signaling pathway	RAP1B
			20	hsa04060: Cytokine-cytokine receptor interaction	IL1RAP
			21	hsa04110: Cell cycle	CCNH
			22	hsa04120: Ubiquitin mediated proteolysis	BRCA1

No.	Gene Symbol	Pathways in KEGG	No.	Pathways in KEGG	Gene Symbol
21	ITGA3	hsa04210: Apoptosis	23	hsa04210: Apoptosis	<i>UBE2D2</i>
		hsa04510: Focal adhesion			<i>BCL2</i>
		hsa04512: ECM-receptor interaction			<i>ILIRAP</i>
22	ITGB1	hsa04640: Hematopoietic cell lineage	24	hsa04310: Wnt signaling pathway	MMPT7
		hsa04810: Regulation of actin cytoskeleton			<i>EFNB1</i>
		hsa05222: Small cell lung cancer			<i>EPHB2</i>
23	LAMB2	hsa04360: Axon guidance	26	hsa04510: Focal adhesion	<i>ITGB1</i>
		hsa04510: Focal adhesion			<i>BCL2</i>
		hsa04512: ECM-receptor interaction			<i>COL4A2</i>
		hsa04514: Cell adhesion molecules (CAMs)			<i>ITGA3</i>
		hsa04670: Leukocyte transendothelial migration			<i>ITGB1</i>
		hsa04810: Regulation of actin cytoskeleton			LAMB2
		hsa05130: Pathogenic Escherichia coli infection – EHEC			<i>PPP1R12A</i>
		hsa05131: Pathogenic Escherichia coli infection – EPEC			<i>RAP1B</i>
		hsa05222: Small cell lung cancer			<i>TNC</i>
23	LAMB2	hsa01430: Cell Communication	27	hsa04512: ECM-receptor interaction	<i>COL4A2</i>
		hsa04510: Focal adhesion			<i>ITGA3</i>
		hsa04512: ECM-receptor interaction			<i>ITGB1</i>
		hsa05222: Small cell lung cancer			LAMB2
24	<i>MAOA</i>	hsa00220: Urea cycle and metabolism of amino groups	28	hsa04514: Cell adhesion molecules (CAMs)	<i>TNC</i>
		hsa00260: Glycine, serine and threonine metabolism			<i>CDH2</i>
		hsa00340: Histidine metabolism			<i>ITGB1</i>

No.	Gene Symbol	Pathways in KEGG	No.	Pathways in KEGG	Gene Symbol
		hsa00350: Tyrosine metabolism			PTPRM
		hsa00360: Phenylalanine metabolism	29	hsa04520: Adherens junction	PTPRM
		hsa00380: Tryptophan metabolism	30	hsa04612: Antigen processing and presentation,	<i>B2M</i>
25	MMP7	hsa04310: Wnt signaling pathway			CANX
26	<i>NDUFA4</i>	hsa00190: Oxidative phosphorylation	31	hsa04640: Hematopoietic cell lineage	CD9
27	<i>NDUFA5</i>	hsa00190: Oxidative phosphorylation			ITGA3
28	POLB	hsa03030: DNA polymerase	32	hsa04670: Leukocyte transendothelial migration	ITGB1
29	<i>PPP1R12A</i>	hsa04510: Focal adhesion			<i>RAP1B</i>
		hsa04720: Long-term potentiation	33	hsa04720: Long-term potentiation	<i>PPP1R12A</i>
		hsa04810: Regulation of actin cytoskeleton			<i>RAP1B</i>
30	PTPRM	hsa04514: Cell adhesion molecules (CAMs)	34	hsa04810: Regulation of actin cytoskeleton	ITGA3
		hsa04520: Adherens junction			ITGB1
31	<i>RAP1B</i>	hsa04010: MAPK signaling pathway			<i>PPP1R12A</i>
		hsa04510: Focal adhesion	35	hsa04940: Type I diabetes mellitus	CPE
		hsa04670: Leukocyte transendothelial migration	36	hsa05030: Amyotrophic lateral sclerosis (ALS)	<i>BCL2</i>
		hsa04720: Long-term potentiation	37	hsa05060: Prion disease	<i>BCL2</i>
		hsa05211: Renal cell carcinoma	38	hsa05130: Pathogenic Escherichia coli infection – EHEC	ITGB1

No.	Gene Symbol	Pathways in KEGG	No.	Pathways in KEGG	Gene Symbol
32	RPL32	hsa03010: Ribosome	39	hsa05131: atrogenic Escherichia coli infection – EPEC	ITGB1
33	SDHC	hsa00020: Citrate cycle (TCA cycle) hsa00190: Oxidative phosphorylation	40	hsa05210: Colorectal cancer	BCL2
34	TNC	hsa01430: Cell Communication hsa04510: Focal adhesion hsa04512: ECM-receptor interaction	41	hsa05211: Renal cell carcinoma	RAP1B
35	TYMS	hsa00240: Pyrimidine metabolism hsa00670: One carbon pool by folate	42	hsa05215: Prostate cancer	BCL2
36	<i>UBE2D2</i>	hsa04120: Ubiquitin mediated proteolysis	43	hsa05222: Small cell lung cancer	BCL2 COL4A2 ITGA3 ITGB1 LAMB2

Bold and Italic symbols respectively indicate the genes up- and down-regulated by GRβ overexpression in both HeLa [10] and U-2 OS cells [11].
* Data from [10].