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REVIEW

Human glucocorticoid receptor isoform β: recent understanding of its potential implications in physiology and pathophysiology

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Abstract The human glucocorticoid receptor (GR) gene expresses two splicing isoforms α and β through alternative use of specific exons 9α and 9β. In contrast to the classic receptor GRα, which mediates most of the known actions of glucocorticoids, the functions of GRβ have been largely unexplored. Owing to newly developed methods, for example microarrays and the jellyfish fluorescence proteins, we and others have recently revealed novel functions of GRβ. 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 GRα-mediated transcriptional activity. A recent report suggested that the "ligand-binding domain" of GRβ is active, forming a functional ligand-binding pocket associated with the synthetic compound RU 486. In this review, we discuss the functions of GRβ, its mechanisms of action, and its pathologic implications.

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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, and the immune/inflammatory response [3, 4]. In addition, glucocorticoids at "pharmacologic" or "stress-related" doses are irreplaceable therapeutic means of treatment of many allergic, inflammatory, autoimmune, and lymphoproliferative diseases [4].

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 is located in chromosome 5 and encodes two splicing variants GRα and GRβ by alternative use of different terminal exons 9α and 9β [5, 7]. GRα is the classic receptor, binding to glucocorticoids and mediating most of the known glucocorticoid actions [5]. In contrast, GRβ does not bind glucocorticoids but functions as a dominant negative inhibitor of GRα-induced transactivation of GRE-containing, glucocorticoid-responsive promoters; its physiologic/pathologic roles have not yet been well elucidated [8, 9].

Using the microarray technique, which enabled us to evaluate gene expression en masse, we and others recently

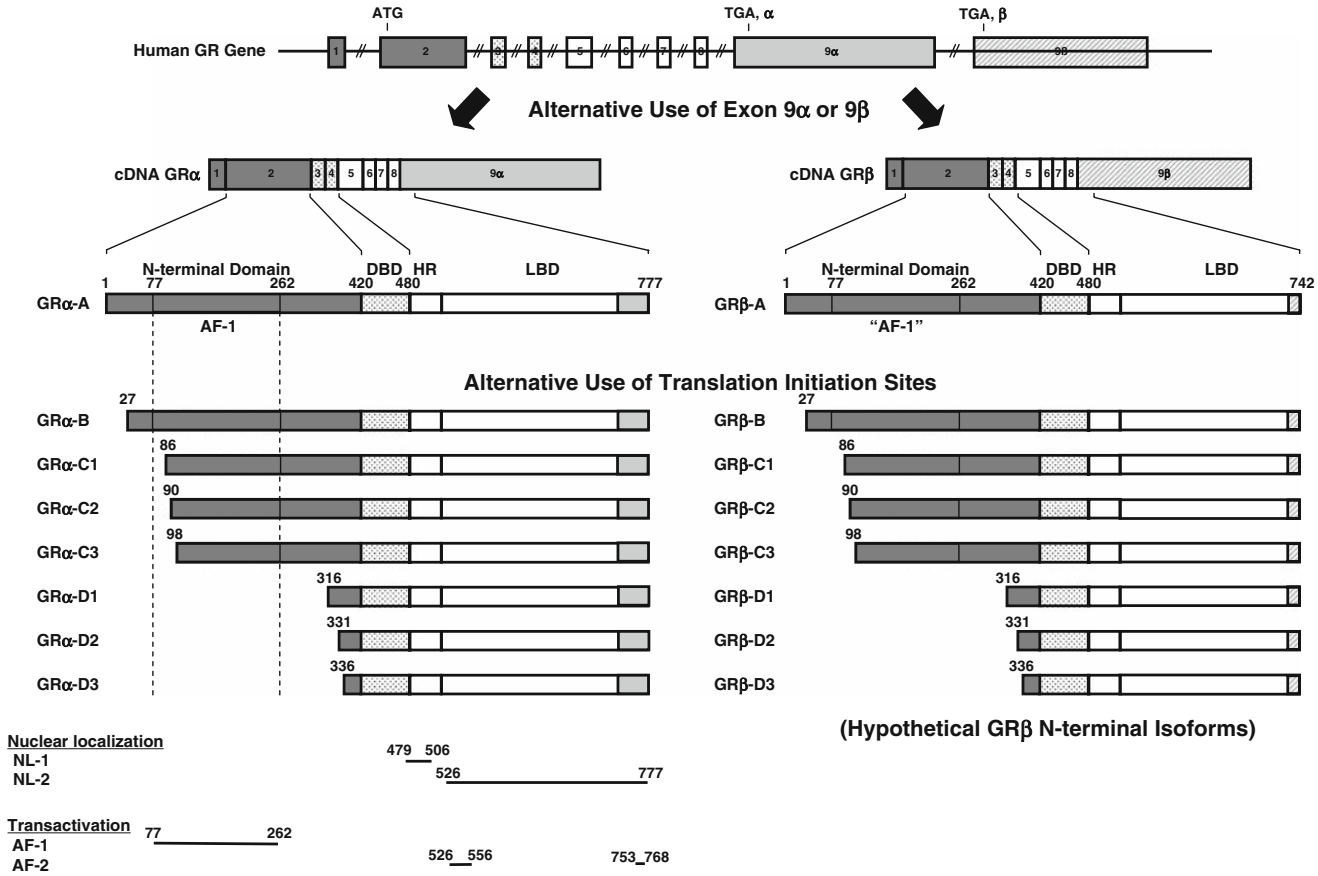

Fig. 1 Genomic and complementary DNA and protein isoforms of the human GR and distribution of functional domains in its linearized molecule. The human GR gene consists of nine exons. Exon 1 is an untranslated region, exon 2 codes for the N-terminal "immunogenic" domain, exons 3 and 4 for the DNA-binding domain, and exons 5–9 for the hinge region and the ligand-binding domain. The GR gene contains two terminal exons 9 (9α and 9β), which produce the classic GRα (GRα-A) and GRβ (GRβ-A) through alternative splicing of these exons. C-terminal *gray colored domains* in GRα-A and GRβ-A show their specific portions. GRα N-terminal translational isoforms expressed from a single GRα transcript are shown in the middle *panel* of the figure. Similar N-terminal translational isoforms may also be produced from the GRβ-specific transcript using the same start sites (modified from Ref. [5]). *AF-1 and -2* activation functions 1 and 2, *DBD* DNA-binding domain, *HD* hinge region, *LBD* Ligand-binding domain, *NL1 and 2* Nuclear translocation signals 1 and 2, *NTD* N-terminal domain

found that the GRβ isoform has intrinsic, GRα-independent transcriptional activity, in addition to its well-known dominant negative effect on GRα [10, 11]. In this review article, we will summarize known GRβ activities and discuss newly identified actions of this GR isoform.

The human GR gene, splicing variants GRα and GRβ, and their multiple translational isoforms

The GR, also known 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 nine 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 9α and/or 9β (Fig. 1). Thus, the GR gene generates ten different transcripts that encode two protein molecules GRα and GRβ.

Recently, it became evident that the GRα variant mRNA is translated from at least eight initiation sites into multiple GRα 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] (Fig. 1). These GR molecules are also differentially expressed in several different cell lines

and tissues [14]. Given that GRα and GRβ share a common mRNA domain that contains the same translation initiation sites [15], it seems that the GRβ variant mRNA is also translated through the same initiation sites to a similar host of eight β isoforms [5] (Fig. 1).

The classic receptor GRα

GRα, the classic glucocorticoid receptor is ubiquitously expressed and mediates most of the known actions of glucocorticoids [3, 5]. The human GRα consists of 777 amino acids and has three major distinct functional domains, the N-terminal or immunogenic domain (NTD), the DNA-binding domain (DBD), and the ligand-binding domain (LBD) [6] (Fig. 1). The LBD of GRα consists of twelve α-helices and four β-sheets, among which helices 3, 4, 11, and 12 form the ligand-binding pocket for binding to glucocorticoids [16–18] (Fig. 2). GRα 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 also [5, 6] (Fig. 3). After binding to its agonist ligand, GRα undergoes conformational changes, dissociates from HSPs, 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 whereas NL-2 spans the entire LBD [19] (Fig. 1).

Inside the nucleus, the ligand-activated GRα 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] (Fig. 3). GR contains two transactivation domains, activation functions (AF)-1 and 2, located at its NTD and LBD, respectively, through which the GR interacts with many proteins and protein complexes, for example 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] (Fig. 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 whereas antagonists, for example RU 486, accumulate the corepressor complexes [22] (Fig. 2).

In addition to transactivation of the glucocorticoid-responsive genes explained above, GRα 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 (Fig. 3). This activity may be more important than the GRE-mediated one, granted that mice harboring a mutant GRα, 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 GRα with other transcription factors may take place on promoters that do not contain GREs (tethering mechanism), and on promoters that have both GRE(s) and responsive element(s) of transcription factors that interact with GRα ("composite promoters") [26]. Repression 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 GRα with nuclear factor-κB (NF-κB), 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, most of these residues are located in the AF-1 domain of the human GR

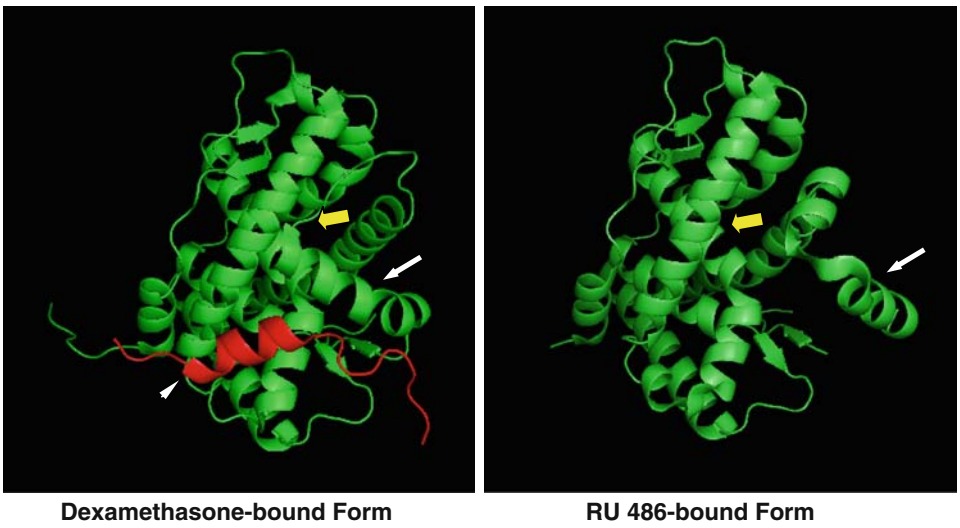

Fig. 2 The three-dimensional structure of GRα associated with agonist dexamethasone (*left*) and antagonist RU 486 (*right*). Results from crystallographic analysis of the GRα associated with agonist dexamethasone (*left*) or with antagonist RU 486 (*right*) are shown [16, 75]. The LBD of GRα consists of twelve α-helices and four 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) whereas 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

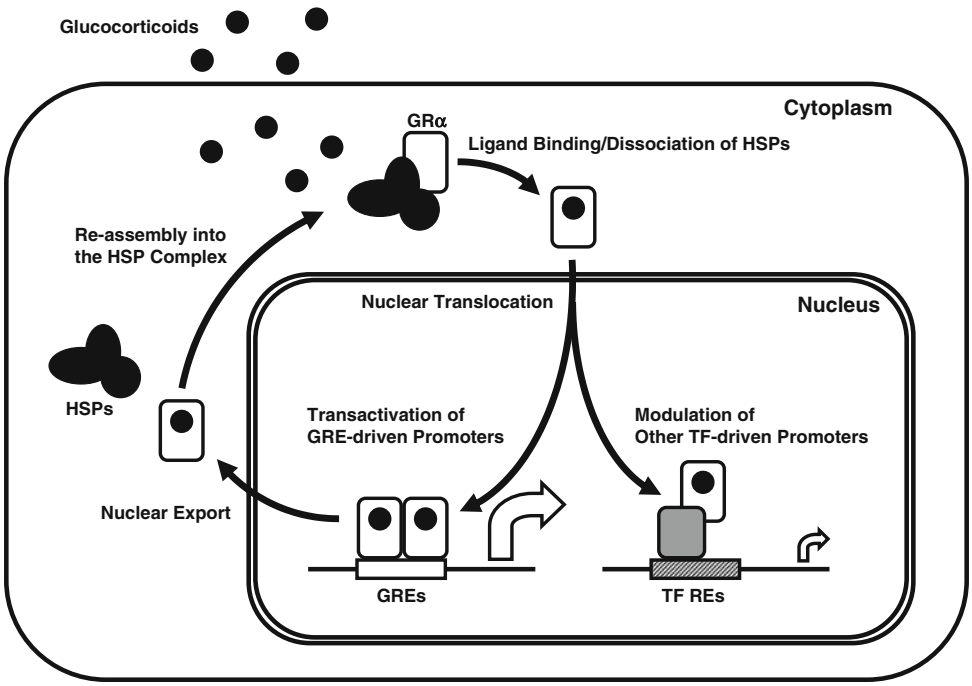

Fig. 3 Nucleocytoplasmic shuttling and transcriptional regulation of GRα. Upon ligand binding, the activated GRα dissociates from the heat-shock proteins (HSPs) and translocates into the nucleus, where it binds as a homodimer to GREs in the promoter regions of target genes or interacts as a monomer with other transcription factors. *GRα* glucocorticoid receptor α, *GRE* glucocorticoid response element, *HSPs* heat-shock proteins, *REs* response elements, *RNPII* RNA polymerase II, *TF* transcription factor

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 for the AF-1 domain of GR [31].

The splicing variant GRβ isoform

Similarly to the classic human GRα, the original human GRβ isoform is also ubiquitously expressed in most tissues. This isoform has been identified in both the zebrafish and humans, but not in mice [15, 32, 33]. The human (h) GRβ contains 742 amino acids and shares the first 727 amino acids from the N-terminus with hGRα [6, 15] (Fig. 1). hGRβ encodes an additional 15 nonhomologous amino acids in the C-terminus, whereas hGRα has an additional 50 amino acids forming a 777-amino-acid protein [6, 15] (Fig. 1). Therefore, hGRβ shares the same NTD and DBD with hGRα, but has a unique "LBD". Because the divergence point (amino acid 727) is located at the C-terminal end of helix 10 in the hGRα LBD, the hGRβ "LBD" does not have helices 11 and 12 of the hGRα. As these helices are important for forming the ligand-binding pocket and for the creation of the AF-2 surface upon ligand binding [16] (Fig. 2), GRβ 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 hGRβ "LBD", the truncated hGR consisting of NTD and DBD is transcriptionally active on GRE-containing promoters [34], thus the hGRβ "LBD" somehow attenuates the transcriptional activity of the other subdomains of the molecule on GRE-driven promoters. Inside cells hGRβ can localize both in the cytoplasm and nucleus [9, 35].

Similarly to the human GR gene, the zebrafish (z) GR gene consists of nine exons and produces the zGRα and zGRβ proteins, which contain 746 and 737 amino acids, respectively [32] (Fig. 4). zGRα and zGRβ share the N-terminal 697 amino acids and have specific C-terminal portions which contain 47 and 40 amino acids, respectively. In contrast to hGRα and hGRβ, which are produced by alternative use of specific exons 9α and 9β, zGRα and zGRβ are formed as a result of intron retention [32]. zGRα and zGRβ use exon 1 to exon 8 for their common N-terminal 697 amino acids. zGRα uses exon 9 for its specific C-terminal portion whereas zGRβ continuously employs the rest of exon 8 and uses a stop codon located at the 3′ portion of this exon to express its specific C-terminal peptide [32] (Fig. 4). Protein alignment comparison of hGRα and zGRβ indicated that these two molecules have exactly the same divergence point and that their β isoform-specific C-terminal peptides show little sequence homology [32]. These pieces of molecular information indicate that hGRβ and zGRβ evolved independently. Nevertheless, zGRβ had the same functional properties as hGRβ, for example inability to bind glucocorticoids, a dominant negative activity on zGRα transcriptional activity on GRE-drive

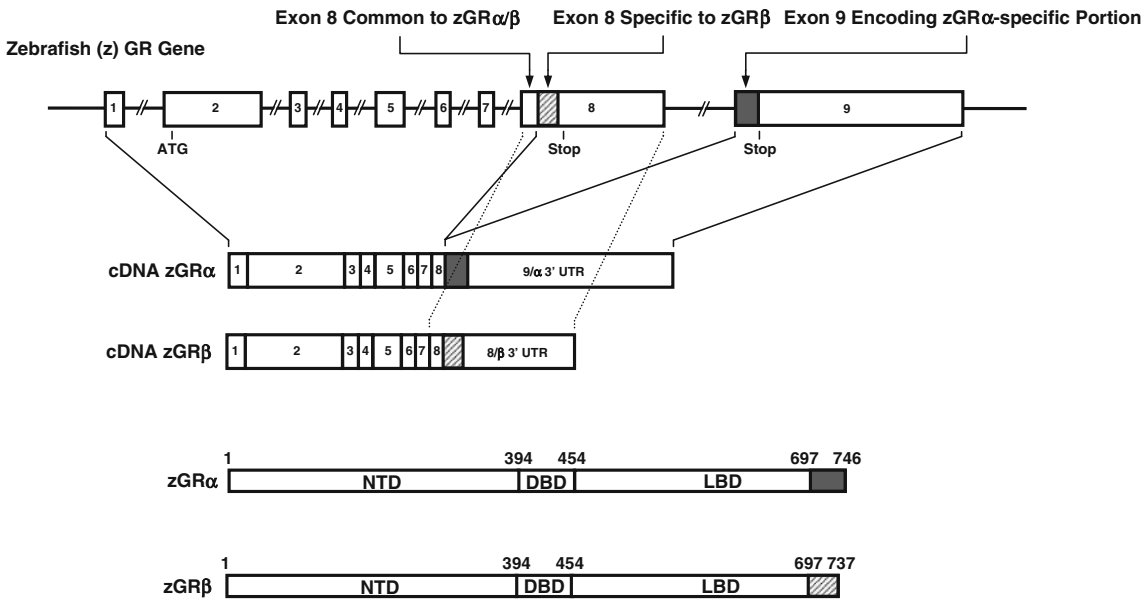

Fig. 4 Genomic and complementary DNA and protein isoforms of the zebrafish GR. The zebrafish (z) GR gene consists of nine exons. The zGR gene expresses zGRα and zGRβ splicing variants through intron retention [32]. C-terminal *gray colored and shaded* domains in zGRα and zGRβ show their specific portions. 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

Table 1 Seventy-eight genes regulated by GRβ overexpression in HeLa and U-2 OS cells observed in two 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	B2 M	*−0.43*	*0.039*	*−0.91*	*0.001*	β2-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, 9 kDa
11	2280	FKBP1A	*−0.32*	*0.030*	*−0.81*	*0.008*	FK506 binding protein 1A, 12 kDa
12	79600	FLJ21127	*−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, β1 polypeptide
15	2123	EVI2A	*−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-MLV 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, 13 kDa
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
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, β1
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, α3
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, α2
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

Table 1 continued

No.	Gene ID	Gene symbol	HeLa Cells*		U-2OS Cells**		Gene name
			Change	*P* value	Change	*P* value	
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
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, β3 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, β2
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 metallopeptidase 7
8	5797	PTPRM	**1.48**	**0.048**	**0.86**	**0.000**	Protein tyrosine phosphatase, receptor type, M

* and ** indicate data from Refs. [10] and [11], respectively (GEO: http:www.ncbi.nlm.nih.gov/geo, GEO Series accession number GSE5310)

Changes are shown in $\log_2$ values

Numbers in bold and italic fonts indicate up-regulation and down-regulation by GRβ overexpression, respectively

promoters, and strikingly similar tissue distribution [32]. Thus, hGRβ and zGRβ are produced by 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, for example the estrogen receptor β (ERβ), thyroid hormone receptor α (TRα), vitamin D receptor, constitutive androstane receptor (CAR), dosage-sensitive sex reversal-1 (DAX-1), nuclear receptor related 2 (Nurr2), neuron-derived orphan receptor-2 (NOR-2), peroxisome proliferator-activated receptor α (PPARα), and PPARγ, 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 GRβ on GRα-induced transcriptional activity: physiologic and pathologic implications

In contrast to GRα, which has numerous and diverse actions [3], the functions of GRβ had not been revealed until we reported its dominant negative effect on GRα-induced transcriptional activity almost a decade after the original identification of this receptor isoform [8]. The dominant negative activity of GRβ 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, GRβ was shown to attenuate glucocorticoid-induced repression of the tumor necrosis factor (TNF) α and interleukin (IL)-6 genes [46]. We also confirmed this negative effect of GRβ on GRα-mediated transrepression using microarray analyses [10]. Several mechanisms explaining this GRβ function have been reported, including:

1 competition for GRE binding through their shared DBD;
2 heterodimerization with GRα; and
3 coactivator squelching through the preserved AF-1 domain [8, 34, 48].

All these different mechanisms of action seem to be functional, depending on the promoters and tissues affected by this GR isoform.

Several clinically oriented investigations suggest that GRβ 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 GRβ, which correlated with reduced sensitivity to glucocorticoids. Elevated levels of pro-inflammatory cytokines, such as IL-1, 2, 4, 7, 8, and 18, TNFα, and interferons α and γ, might have been responsible for increased GRβ expression in cells from patients with these pathologic conditions, because these cytokines experimentally stimulated expression of GRβ in lymphocytes, neutrophils, or airway smooth-muscle cells [56–61]. Further, the presence of a single nucleotide polymorphism in the 3′ untranslated region of the hGRβ mRNA (rs6198G allele), which increases the stability of the mRNA, and thus, causes elevated expression of GRβ protein, was associated with increased incidence of RA, SLE, high blood pressure, ischemic heart diseases, and nasal carriage of *Staphylococcus aureus* [50, 62–64],

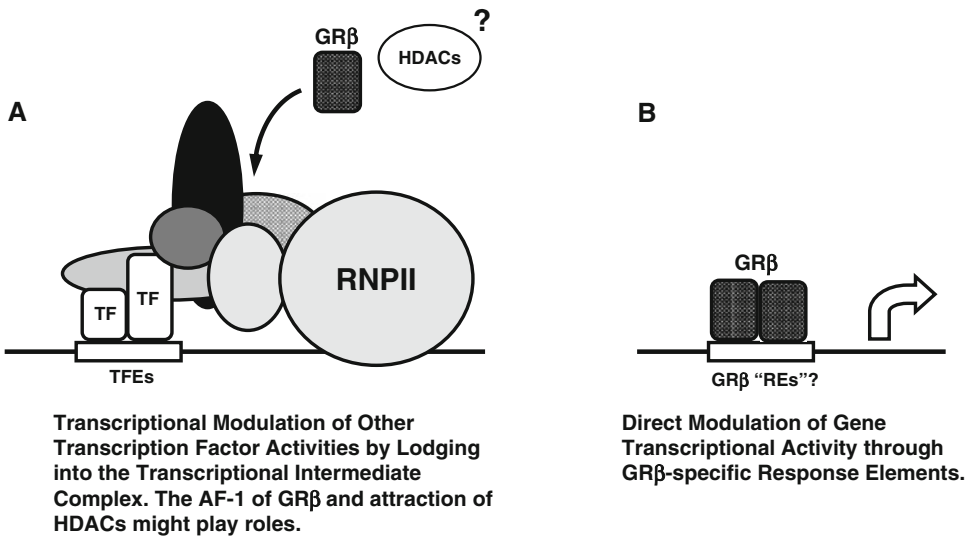

Fig. 5 Hypothetical models for GRβ-mediated modulation of the transcriptional activity of its responsive genes. **a** Through AF-1 located in the NTD, GRβ may interact with numerous transcriptional cofactors and transcriptional factors, lodge into the transcription intermediate complex formed on the promoter region of GRβ-responsive genes, and modulate their transcriptional activity. GRβ may attract histone deacetylases to the transcription intermediate complex formed on the promoter region of genes regulated by this GR isoform. **b** GRβ might also bind to hypothetical specific response elements located in the promoter region of responsive genes, directly modulating their transcriptional activity. *GRβ* glucocorticoid receptor β, *HDACs* histone deacetylases, *REs* response elements, *RNPII* RNA polymerase II, *TF* transcription factor

Table 2 Thirty-six genes regulated by GRβ overexpression in HeLa cells are involved in 43 distinct biologic pathways in KEGG

No.	Gene symbol	Pathways in KEGG	No.	Gene symbol	Pathways in KEGG
1	ABCA3	hsa02010: ABC transporters—General	1	SDHC	hsa00020: Citrate cycle (TCA cycle)
2	**ACY1**	hsa00220: Urea cycle and metabolism of amino groups	2	*NDUFA4*	hsa00190: Oxidative phosphorylation
3	*B2 M*	hsa04612: Antigen processing and presentation,		*NDUFA5*	
4	*BCL2*	hsa01510: Neurodegenerative Diseases		SDHC	
		hsa04210: Apoptosis	3	**ACY1**	hsa00220: Urea cycle and metabolism of amino groups
		hsa04510: Focal adhesion		*MAOA*	
		hsa05030: Amyotrophic lateral sclerosis (ALS)	4	*DUT*	hsa00240: Pyrimidine metabolism
		hsa05060: Prion disease		**TYMS**	
		hsa05210: Colorectal cancer		DTYMK	
		hsa05215: Prostate cancer	5	GCLC	hsa00251: Glutamate metabolism
		hsa05222: Small cell lung cancer	6	*MAOA*	hsa00260: Glycine, serine and threonine metabolism
5	BRCA1	hsa04120: Ubiquitin mediated proteolysis	7	*MAOA*	hsa00340: Histidine metabolism
6	CANX	hsa04612: Antigen processing and presentation,	8	*FAH*	hsa00350: Tyrosine metabolism
7	*CCNH*	hsa04110: Cell cycle		*MAOA*	
8	**CD9**	hsa04640: Hematopoietic cell lineage	9	*MAOA*	hsa00360: Phenylalanine metabolism
9	CDH2	hsa04514: Cell adhesion molecules (CAMs)	10	*MAOA*	hsa00380: Tryptophan metabolism
10	COL4A2	hsa01430: Cell Communication	11	GCLC	hsa00480: Glutathione metabolism
		hsa04510: Focal adhesion		HYAL2	hsa00531: Glycosaminoglycan degradation
		hsa04512: ECM-receptor interaction		*FAH*	hsa00643: Styrene degradation
		hsa05222: Small cell lung cancer		**TYMS**	hsa00670: One carbon pool by folate
11	CPE	hsa04940: Type I diabetes mellitus	12	HYAL2	hsa01032: Glycan structures—degradation
12	DTYMK	hsa00240: Pyrimidine metabolism,	13	COL4A2	hsa01430: Cell Communication
13	*DUT*	hsa00240: Pyrimidine metabolism		**LAMB2**	
14	EFNB1	hsa04360: Axon guidance		TNC	
15	EPHB2	hsa04360: Axon guidance	14	*BCL2*	hsa01510: Neurodegenerative Diseases
16	*FAH*	hsa00350: Tyrosine metabolism	15	ABCA3	hsa02010: ABC transporters—General
		hsa00643: Styrene degradation	16	RPL32	hsa03010: Ribosome
17	GCLC	hsa00251: Glutamate metabolism	17	*GTF2A2*	hsa03022: Basal transcription factors
		hsa00480: Glutathione metabolism	18	POLB	hsa03030: DNA polymerase
18	*GTF2A2*	hsa03022: Basal transcription factors	19	*RAP1B*	hsa04010: MAPK signaling pathway
19	HYAL2	hsa00531: Glycosaminoglycan degradation	20	IL1RAP	hsa04060: Cytokine–cytokine receptor interaction
		hsa01032: Glycan structures—degradation	21	*CCNH*	hsa04110: Cell cycle
20	IL1RAP	hsa04060: Cytokine–cytokine receptor interaction	22	BRCA1	hsa04120: Ubiquitin mediated proteolysis
		hsa04210: Apoptosis		*UBE2D2*	
21	ITGA3	hsa04510: Focal adhesion	23	*BCL2*	hsa04210: Apoptosis
		hsa04512: ECM-receptor interaction		IL1RAP	
		hsa04640: Hematopoietic cell lineage	24	**MMP7**	hsa04310: Wnt signaling pathway
		hsa04810: Regulation of actin cytoskeleton	25	EFNB1	hsa04360: Axon guidance
		hsa05222: Small cell lung cancer		EPHB2	
22	ITGB1	hsa04360: Axon guidance		ITGB1	
		hsa04510: Focal adhesion	26	*BCL2*	hsa04510: Focal adhesion
		hsa04512: ECM-receptor interaction		COL4A2	
		hsa04514: Cell adhesion molecules (CAMs)		ITGA3	
		hsa04670: Leukocyte transendothelial migration		ITGB1	
		hsa04810: Regulation of actin cytoskeleton		**LAMB2**	
		hsa05130: Pathogenic *Escherichia coli* infection—EHEC		*PPP1R12A*	
		hsa05131: Pathogenic *Escherichia coli* infection—EPEC		*RAP1B*	
		hsa05222: Small cell lung cancer		TNC	

Table 2 continued

No.	Gene symbol	Pathways in KEGG	No.	Gene symbol	Pathways in KEGG
23	**LAMB2**	hsa01430: Cell Communication	27	COL4A2	hsa04512: ECM-receptor interaction
		hsa04510: Focal adhesion		ITGA3	
		hsa04512: ECM-receptor interaction		ITGB1	
		hsa05222: Small cell lung cancer		**LAMB2**	
24	*MAOA*	hsa00220: Urea cycle and metabolism of amino groups		TNC	
		hsa00260: Glycine, serine and threonine metabolism	28	CDH2	hsa04514: Cell adhesion molecules (CAMs)
		hsa00340: Histidine metabolism		ITGB1	
		hsa00350: Tyrosine metabolism		**PTPRM**	
		hsa00360: Phenylalanine metabolism	29	**PTPRM**	hsa04520: Adherens junction
		hsa00380: Tryptophan metabolism	30	*B2M*	hsa04612: Antigen processing and presentation,
25	**MMP7**	hsa04310: Wnt signaling pathway		CANX	
26	*NDUFA4*	hsa00190: Oxidative phosphorylation	31	**CD9**	hsa04640: Hematopoietic cell lineage
27	*NDUFA5*	hsa00190: Oxidative phosphorylation		ITGA3	
28	POLB	hsa03030: DNA polymerase	32	ITGB1	hsa04670: Leukocyte transendothelial migration
29	*PPP1R12A*	hsa04510: Focal adhesion		*RAP1B*	
		hsa04720: Long-term potentiation	33	*PPP1R12A*	hsa04720: Long-term potentiation
		hsa04810: Regulation of actin cytoskeleton		*RAP1B*	
30	**PTPRM**	hsa04514: Cell adhesion molecules (CAMs)	34	ITGA3	hsa04810: Regulation of actin cytoskeleton
		hsa04520: Adherens junction		ITGB1	
31	*RAP1B*	hsa04010: MAPK signaling pathway		*PPP1R12A*	
		hsa04510: Focal adhesion	35	CPE	hsa04940: Type I diabetes mellitus
		hsa04670: Leukocyte transendothelial migration	36	*BCL2*	hsa05030: Amyotrophic lateral sclerosis (ALS)
		hsa04720: Long-term potentiation	37	*BCL2*	hsa05060: Prion disease
		hsa05211: Renal cell carcinoma	38	ITGB1	hsa05130: Pathogenic *Escherichia coli* infection—EHEC
32	RPL32	hsa03010: Ribosome	39	ITGB1	hsa05131: Pathogenic *Escherichia coli* infection—EPEC
33	SDHC	hsa00020: Citrate cycle (TCA cycle)	40	*BCL2*	hsa05210: Colorectal cancer
		hsa00190: Oxidative phosphorylation	41	*RAP1B*	hsa05211: Renal cell carcinoma
34	TNC	hsa01430: Cell Communication	42	*BCL2*	hsa05215: Prostate cancer
		hsa04510: Focal adhesion	43	*BCL2*	hsa05222: Small cell lung cancer
		hsa04512: ECM-receptor interaction		COL4A2	
35	**TYMS**	hsa00240: Pyrimidine metabolism		ITGA3	
		hsa00670: One carbon pool by folate		ITGB1	
36	*UBE2D2*	hsa04120: Ubiquitin mediated proteolysis		**LAMB2**	

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

possibly through inhibition of glucocorticoid actions by increased concentrations of GRβ. These pieces of clinical evidence further support the dominant negative activity of GRβ on GRα-induced transcription inside the human body, functioning as a negative regulator of glucocorticoid actions in local tissues.

GRβ has intrinsic, GRα-independent transcriptional activity

We and others recently performed transcriptome analyses using microarray techniques in cultured cells overexpressing GRβ, and found that these cells had a distinct mRNA expression profile compared with cells not overexpressing GRβ and those expressing GRα and treated with glucocorticoids [10, 11]. In a subsequent real-time PCR analysis, we also confirmed that GRβ regulates mRNA expression positively and negatively in a gene-specific fashion [10]. These results indicate that GRβ has intrinsic transcriptional activities independent of the activity of its isoform GRα. We have compared the microarray results obtained by us and those of others [10, 11], and found that the two studies share 78 genes modulated by overexpression of GRβ (Table 1). Specifically, 29 out of 78 genes were both down-regulated by GRβ overexpression, whereas only eight were up-regulated.

Interestingly, 41 genes showed opposite response to GRβ between the two studies, suggesting that GRβ modulates mRNA expression of some of its responsive genes in a cell-specific and, possibly, cell culture condition-specific fashion.

Apparently, this intrinsic transcriptional activity of GRβ is not mediated by binding of the isoform to classic GREs, as GRβ does not affect the transcriptional activity of classic GRE-driven promoters, whereas the promoter regions of the genes, which we identified to be regulated by GRβ, do not contain GRE sequences [10]. Rather, GRβ 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 GRβ, which presumably keeps the same protein structure and function as that of GRα, is transcriptionally active, contributing to its dominant negative activity against GRα-induced transactivation [34]. This transactivation domain of GRα 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 GRβ alters the transcriptional activity of its responsive genes by lodging into the transcriptional complexes formed on their promoter region through its AF-1 (Fig. 5). This mechanistic hypothesis is further supported by recent results from other groups, which showed GRβ repressed the transcriptional activity of AP-1 and NFκB, possibly through protein–protein interactions similar to those between GRα and these transcription factors [71].

GRβ 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, GRβ might bind DNA sequences unique to this isoform through its DBD, regulating transcription through hypothetical "GRβ REs" (Fig. 5). Because the subdomains of steroid hormone receptors affect each others' activities [73, 74], the unique GRβ "LBD" might alter the binding specificity of its DBD to DNA and allow it to recognize a set of DNA sequences specific to GRβ and distinct from those of GRα.

The importance and exact roles of this intrinsic transcriptional activity of the GRβ isoform in physiology and pathophysiology have not yet been elucidated. We have performed a pathway analysis of our microarray results to define the biologic pathways where GRβ 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, GRβ might strongly affect 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), and the metabolism of some amino acids and other bioactive molecules. Interestingly, GRβ 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 GRβ plays important roles, development of mice conditionally over-expressing human GRβ would be very helpful.

Issues on "ligand" and subcellular localization of GRβ

A previous publication demonstrated that only RU 486 among 57 native and synthetic steroids tested bound GRβ weakly at the "ligand-binding" pocket of the GRβ and slowly (over 6 h for completion) induced its nuclear translocation [11]. The results were supported by a nuclear translocation study using fluorescent protein-fused GRβ 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 GRβ "ligand-binding domain" associated with several steroids [11]. This report also demonstrated that RU 486 modulated GRβ-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 GRβ. Crystallographic structural analysis of the GRβ "LBD" might help identifying a "ligand-binding pocket" in the GRβ "LBD" and hence its binding to RU 486. The cytoplasmic to nuclear translocation of GRβ demonstrated by the previous work was quite slow compared with that of GRα: in the former, the receptor took 6 h to complete its translocation whereas in the latter it did this within minutes [11]. GRβ and GRα share NL-1, which mediates the rapid nuclear translocation of GRα, whereas GRβ does not appear to have NL-2, which is dependent on the entire LBD of GRα, and causes slower nuclear translocation of the receptor [19]. Thus, the presence of yet unknown regulators specific to GRβ might be involved in the nuclear translocation of this isoform.

We independently performed several experiments addressing the potential activation of GRβ by RU 486, its subcellular localization, and cytoplasmic to nuclear translocation. In contrast to the previously reported findings [11], the green fluorescent protein-fused GRβ was mainly located in the nuclei of HeLa cells stably expressing this fusion protein, whereas it was heterogeneously distributed both in the cytoplasm and the nucleus in HCT116 cells that expressed the GRβ fusion protein transiently: some cells mainly expressed GRβ in the nucleus whereas 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 GRβ-expressing HCT116 cells, and did not induce cytoplasmic to nuclear translocation of this isoform [10]. The inconsistency of our results with those previously reported may have been caused by use of different experimental systems, for example cell lines and plasmids. This discrepancy suggests that the mechanisms of the regulatory actions of GRβ on the transcription of responsive genes inside the cells are quite complex.

Summary

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

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