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The Interplay between the Glucocorticoid Receptor Activity and post-Translational Modifications in the Immune and Neuroendocrine Systems

María Antunica-Noguerol[a,b], Fernando Aprile-Garcia[a,b], Maia Ludmila Budziñski[a], Luciano Proto-Cassina[a], Ana C. Liberman[a,1] and Eduardo Arzt[a,b,*,1]
[a]*Instituto de Investigación en Biomedicina de Buenos Aires – CONICET – Partner Institute of the Max Planck Society, Buenos Aires, Argentina*
[b]*Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina*

Abstract. Glucocorticoids (GCs), the most downstream effectors of the hypothalamic-pituitary-adrenal (HPA) axis, are key mediators in the interaction between immune and neuroendocrine systems. They exert their biological actions mainly through binding to their intracellular receptor, the glucocorticoid receptor (GR), which in turn influences gene expression by interacting with transcription factors and/or coregulators. GR abnormal function has been extensively associated to stress-related disorders, inflammatory and autoimmune diseases. Therefore, modulating GR activity is critical to overcome pathological conditions. The final outcome of GCs actions in the immune and neuroendocrine systems is regulated at multiple levels, including post-translational modifications (PTMs) of GR as well as of protein complexes involved in GR signaling. Understanding the influence of PTMs on the molecular mechanisms involved in GR signaling is thus of utmost importance in the search for therapeutic strategies aimed at modulating GR responses under pathophysiological circumstances, and to understand the neuroimmune circuits.

Keywords: Glucocorticoid receptor, post-translational modifications, transcription regulation

GLUCOCORTICOIDS IN THE IMMUNE AND NEUROENDOCRINE SYSTEM

Glucocorticoids

The hypothalamic-pituitary-adrenal (HPA) axis plays a fundamental role in the response to external and internal stimuli. Activation of the HPA axis is a tightly regulated process that occurs both in a circadian way and in response to stress. Upon activation of the HPA axis, two neuropeptides, corticotropin-releasing hormone (CRH) and vasopressin (AVP), are released by the parvocellular neurons of the hypothalamic paraventricular nucleus (PVN) into the portal vessel system to activate the synthesis of propiomelanocortin (POMC) in the anterior pituitary. POMC is then processed to corticotropin (ACTH), among other peptides; ACTH then stimulates the adrenal cortex to secrete glucocorticoids (GCs) into the blood stream -cortisol in humans

[1]These authors contributed equally to this work.

*Correspondence to: Dr. Eduardo Arzt, Instituto de Investigación en Biomedicina de Buenos Aires, Godoy Cruz 2390, C1425FQD, Buenos Aires, Argentina. Tel.: +54 11 4899 5500; E-mail: earzt@ibioba-mpsp-conicet.gov.ar.

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and corticosterone in roedents- [1]. GCs are therefore the most downstream effectors of the HPA axis and will trigger different responses depending on cellular context and target tissue. As a consequence, GCs exhibit a wide spectrum of biological activities that range from metabolic to the well-characterized anti-inflammatory and immunosuppressive actions.

GCs are corticosteroid hormones that share a common precursor, cholesterol, with progestins, androgens and estrogens. They are naturally occurring small lipophilic compounds that display a broad range of actions during stress responses, including regulation of the cardiovascular tone, fluid volume and responses to hemorrhage, immunity and inflammation, metabolism, neural function and behavior, and reproduction [2]. In particular, extensive attention has been given to the effects of GCs in the regulation of the immune system and immune responses, since GCs are the most potent anti-inflammatory and immunosuppressive drugs known so far. Their ability to down-regulate inflammatory processes mainly relies in the inhibition of the expression of multiple inflammatory genes -encoding cytokines, chemokines, adhesion molecules and inflammatory enzymes- and their biological actions. In turn, inhibition of the expression of such inflammatory molecules culminates in the alteration of immune and non-immune cells functions involved in this response. GCs alter the activity of both non-lymphoid inflammatory cells, such as macrophages, neutrophils, basophils, eosinophils, mast cells, endothelial cells and fibroblasts, as well as lymphoid cells (T and B cells), by down-regulating their activity and/or activation [3, 4]. Since GCs are the ultimate connection between the HPA axis and the immune system, understanding the fine-tuning of GCs actions is crucial.

GCs are extensively used in the pharmacological treatment of inflammatory, allergic and autoimmune disorders and in the treatment of transplanted patients to avoid organ rejection. However, their use is often accompanied by undesirable side effects. These side effects result from targeting different organs and systems, apart from the immune system, including skin; skeleton and muscles; eye; gastrointestinal tract; central nervous system (CNS) and endocrine, cardiovascular and immune systems. Severity of these side effects varies from mild, like hypertrichosis – enhanced hair growth-, to extreme and even life threatening, like gastric hemorrhage [5]. Therefore, and even though much effort has been spent on this matter, the search for drugs bearing strong immunomodulatory and immunosuppressive actions like GCs with reduced or no collateral effects is still an unresolved issue. Deciphering the molecular mechanisms that underlie GCs actions is critical to achieve this goal.

Glucocorticoid receptor

Due to their lipophilic nature, GCs diffuse freely through the cell membrane into the cytoplasm where they exert their main actions by binding to their intracellular receptor. The glucocorticoid receptor (GR) is a hormone-activated transcription factor (TF) that belongs to the nuclear steroid/thyroid receptor superfamily [6].

Like many other nuclear receptors, GR is a modular protein that consists of an N-terminal domain, encompassing a transactivation domain called activation function 1 (AF-1) that is responsible for transcriptional activation and association with certain basal transcription factors; a DNA-binding domain (DBD), which bears a dimerization domain that plays a role in GR dimerization and DNA-binding; and a C-terminal ligand-binding domain (LBD), containing a second transactivation domain (AF-2) regulated by hormone binding [7]. The LBD also encompasses a dimer interface critical for GR function [8] and a heat-shock protein (Hsp) 90 binding site. Interestingly, point mutations within the LBD alter the activities of bound coregulators, which are critical for GR-mediated transcription -as it will be discussed later- without altering their binding [9].

GR activation is tightly regulated by a dynamic multiprotein complex. This chaperone complex includes heat-shock proteins, such as Hsp90 and Hsp70, and immunophilins, such as the FK506 binding protein (FKBP) 51 and FKBP52 [10]. Upon ligand binding, GR undergoes a conformational change that promotes its translocation to the nucleus where it recruits regulatory cofactor complexes and influences gene transcription [11]. Translocation to the nucleus is a dynamic process; GR retains the ability to shuttle between the nucleus and the cytoplasm and is not statically retained into the nucleus [12]. Against previous beliefs, it is now accepted that GR does not dissociate from its chaperone complex when binding to its ligand but instead remains associated within this complex to translocate to the nucleus. Indeed, integrity of the chaperone complex seems to be critical for GR nuclear translocation [13, 14]. The relevance of Hsp90 on GR activity has been extensively documented [10, 15–17]. In particular, pharmacological disruption of Hsp90 function has proven to be a useful tool to shed light upon the importance of Hsp90

in regulating immune and neuroendocrine responses in a GR-dependent manner. Inhibition of Hsp90 was found to interfere with the anti-inflammatory actions of ligand-activated GR [18, 19], apparently by attenuating GR inhibition of pro-inflammatory TFs [20]. Moreover, inhibition of Hsp90 chaperoning function in a neuroblastoma cell line leads to reduced GR transactivation by interfering with GR-Hsp90 association, and induced-GR proteasomal degradation [21]. It also impairs GR retrograde movement in neurites from NT2 stem cells differentiated into neuronal phenotype, while inducing GR degradation as well [22]. In addition, altering GR-Hsp90 interaction impacts on stress-related behavior *in vivo* [23–25].

Not only Hsp90 but also other components of the GR-Hsp90 heterocomplex play critical roles in regulating GR function. On this matter, it was recently reported that immunophilin composition of the GR chaperone complex can either enhance or repress GR translocation, since FKBP51 delays GR nuclear transport while FKBP52 binding to dynein appears to be responsible for FKBP52 mediated enhancement of GR nuclear translocation [26, 27]. Interestingly, FKBP51 gene (*FKBP5*) expression is up-regulated by ligand-activated GR through distant-acting enhancers [28], suggesting the existence of an ultra-short negative feedback loop regulating GR activation. Alterations in both FKBP51 and FKBP52 have been implicated in impaired GR signaling and stress-related disorders associated to HPA axis disfunction. On one hand, polymorphisms in the co-chaperone *FKBP5* gene have been associated to differential up-regulation of FKBP51 following GR activation and differences in GR sensitivity and stress hormone system regulation. Enhanced expression of FKBP51 following GR activation leads to an increased GCs resistance and decreased efficiency of the negative feedback loop. This deregulated stress response might be a risk factor for stress-related psychiatric disorders [29]. Moreover, FKBP51 has also been associated to immune-related diseases and inflammation. Its role in these pathologies is apparently mediated not only by its cochaperone function but also by its ability to modulate nuclear factor-kB (NF-kB) dependent gene expression [30]. On the other hand, FKBP52 has also been proposed as a therapeutic target based on results obtained in *in vivo* experiments with knockout mice [31]. In particular, heterozygous FKBP52 knockout mice were found to display an altered phenotype regarding behavioral parameters and neuroendocrine levels under basal and chronic stress conditions. Alteration in these parameters is most likely due to reduced GR sensitivity [32], highlighting the importance of FKBP52 function on GR activity.

Mechanism of action

Once in the nucleus, GR can regulate transcription of target genes either in a positive or negative fashion. GR transcriptional activation is mainly achieved by GR binding as a homodimer to specific palindromic DNA consensus sequences, the glucocorticoid response elements (GREs). By this mechanism of transactivation, GR positively regulates target genes bearing GREs in their regulatory regions [3]. Recently, it has been demonstrated that GR binding mostly occurs at pre-existing regions of open chromatin, therefore postulating chromatin structure as a plausible mechanism for the observed cell-selective GR occupancy patterns [33, 34]. It has also been described that GR can positively influence gene transcription by binding to the so-called composite sites, where GREs and other TF binding sites are involved [11].

On the other hand, transcriptional repression of target genes is mainly achieved by GR protein – protein interaction with other TFs. By this mechanism of transrepression, GR interferes with other TFs activities such as activating protein-1 (AP-1), NF-kB and T-box expressed in T cells (T-bet) [35, 36]. GR does not directly bind to DNA but is instead recruited to other TFs binding sites, called tethering elements, through direct protein physical interaction [3, 37].

Negative regulation of target genes can also be achieved by GR DNA binding through interaction of monomeric GR with negative GREs (nGRE), even though it has not been considered until recently as a widespread mechanism for GR transcriptional repression. A genome-wide conserved family of palindromic nGREs has been identified to mediate transrepression by direct binding of ligand-bound GR and sequential assembly of a repressing complex [38]. Taking the thymic stromal lymphopoetin (TSLP) promoter as a model, it was demonstrated that GR binds to the nGREs along this promoter in such a conformation that prevents DNA-mediated dimerization, in contrast to the DNA-mediated dimerization found on activating GREs. Binding to these nGRE sequences alters the conformation of GR residues that are critical for transcriptional activation so that negative regulation is accomplished [39].

Activation or repression of target genes is achieved by GR recruitment of coregulators that serve as coactivators or corepressors, respectively, to responsive regulatory regions [40, 41]. GR ligands with agonistic

or antagonistic properties apparently induce a particular GR conformation that allows for the recruitment of coactivators or corepressors to the complex, thereby modulating the final outcome. Ligand-selective interaction of the GR with different coregulators may provide a feasible biochemical explanation for ligand-selective and promoter-specific differences observed in both transactivation and transrepression mechanisms [42–44].

Coactivators include a wide range of proteins that enhance nuclear receptor-dependent transcription through interaction with the ligand-bound receptor. They generally mediate interaction between nuclear receptors and the general transcription machinery. In addition, most of the coactivators also display enzymatic activities that contribute to their function in promoting transcription, such as histone acetyl-transferase (HAT) and histone methyl-transferase. They mediate chromatin remodeling and facilitate the association of RNA polymerase II complex with the general transcription machinery at the promoter of the target gene [45]. Competition for coactivator recruitment was initially proposed as an explanation for transcriptional repression exerted by nuclear receptors. It was hypothesized that competition for limiting amounts of coactivators that are common to many intracellular signaling pathways may account, at least partly, for GR inhibitory effects on other TFs activities. In particular, both AP-1 and GR recruit cAMP response element binding protein (CREB)-binding protein (CBP) and p300 upon activation. Therefore, competition for relatively low intracellular levels of CBP/p300 was proposed to explain AP-1 transcriptional inhibition in the presence of ligand-activated GR [46]. However, this hypothesis was later discarded. An updated study on GR-dependent transrepression of AP-1 showed that GCs can mediate suppression of the AP-1-driven interleukin (IL)-6 promoter and other AP-1 driven genes independently of the levels of coexpressed coactivators [47]. As for AP-1, it was also demonstrated that GR-mediated transrepression of other inflammatory TFs, such as NF-kB, occurs regardless of coactivators levels [48]. Interestingly, it has been demonstrated that GR tethered at AP-1 and NF-kB target sites through protein interaction does not compete but instead alters coactivator recruitment required for gene activation, resulting in diminished gene expression. For example, interaction of NF-kB with both coactivators interferon regulatory transcription factor (IRF) 3 and the positive transcription elongation factor b (PTEFb) is impeded by GR tethered to NF-kB-binding site, thereby inhibiting gene expression [49].

Nevertheless, the occurrence of active transrepression mechanisms should not be disregarded. Recruitment of corepressors by unliganded or antagonist-bound nuclear receptors partly accounts for inhibition of gene expression. Two major corepressors identified to interact with GR are nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptor (SMTR) [50]. An interesting example arises from glucocorticoid receptor interacting protein (GRIP) 1, which is recruited by GR upon ligand binding. GRIP1 belongs to the p160/steroid receptor coactivator (SRC) family of coregulators [51]. It has been demonstrated that GRIP1, apart from its coactivator activity, displays corepressor activity when recruited to GR tethered AP-1 target sites. This inhibitory effect of GRIP1 was not mimicked by other members of the p160 coactivator family. Indeed, GRIP1 corepressor activity mapped to a region of the protein with no evident similarity to the other members' sequences and was specific of the GR repressive complex built at AP-1 tethering site [52]. A recent study on GR-regulation of LPS-stimulated macrophages gene expression showed that GRIP1 is equally recruited to both up- and down-regulated genes [53]. Mechanisms switching GRIP1 from coactivator to corepressor or viceversa, depending on the context, remain yet to be elucidated.

Other corepressors that are recruited by GR to transrepressed genes are histone deacetylases (HDACs). Opposite to HAT activity that is generally associated with transcriptional activation, HDAC activity is widely linked to transcriptional silencing [54]. Therefore, recruitment of HDACs to GR negatively regulated genes contributes to inhibit their expression. The best documented example in this regard is HDAC2. Activated GR targets this deacetylase to inflammatory gene promoters in order to suppress their activity [37, 55]. Moreover, HDAC activity appears to be important for transrepression not only regarding immunosuppressive and anti-inflammatory actions of GCs but also at the neuroendocrine level. In particular, HDAC2 is involved in GR mediated repression of POMC gene expression. POMC is down-regulated by ligand-activated GR as part of the negative feedback loop regulating the secretion of GCs under stress. Upon GC stimulation both GR and HDAC2 are recruited to POMC promoter leading to a reduction in histone acetylation [56]. Interestingly, HDAC3 has been suggested as a putative mediator in GR negative transcriptional feedback over its own promoter, since ligand-induced repression of GR gene transcription is mediated by assembly of a GR, NCoR1 and HDAC3-containing repression

complex at the proximal promoter region of the GR gene [57]. Recently, acetylation of histone H3 was shown to be reduced upon GC treatment in repressed enhancers of both unstimulated and LPS-stimulated macrophages and this decrement was found to correlate with enrichment in HDAC occupancy [53].

Surprisingly, HDACs have also been associated to GR-dependent transcriptional activation. It was first demonstrated that treatment with a HDAC inhibitor prevented GCs-induced expression of the mouse mammary tumor virus (MMTV) [58]. Accordingly, HDAC1 was shown to be required for ligand-induced GR-dependent MMTV promoter activation. HDAC1 is recruited to the GR coactivator complex. In turn, after an initial peak of increase in the promoter activity, the promoter is subjected to rapid suppression. This event correlates with inactivation of HDAC1 within the complex. Therefore, HDAC1 deacetylase activity appears to be critical for GR-dependent MMTV-promoter activation [59]. Together with HDAC1, HDAC2 was also found to be critical for GR-mediated transactivation, since knockdown of HDAC1 or HDAC2 separately decreased MMTV transcription. Furthermore, knockdown of both deacetylases had a synergistic effect on MMTV transcription [60]. A wider approach later demonstrated that a HDAC inhibitor significantly reduced GR-transactivation at half of GCs-induced analyzed genes, by inhibiting GCs-induced transcription. In this study, HDAC1 was also identified to be a major player in GCs-induced transactivation [61]. Therefore, HDAC involvement in GR-mediated transcription seems to have genome-wide effects and the final outcome should be carefully considered.

Glucocorticoids actions in the immune and neuroendocrine system

The neuroendocrine system regulates the immune response through several pathways, including the HPA axis, the hypothalamic-pituitary-thyroid axis, the hypothalamic-pituitary-gonadal axis, and the hypothalamic-growth hormone axis [62]. The connection between the neuroendocrine system and the immune system provides a finely tuned regulatory mechanism for homeostasis. Signaling from the immune system to the HPA axis was first reported almost thirty years ago, when IL-1 was the first cytokine to be identified as an afferent signal mediating immunoregulatory feedback through the neuroendocrine system [63, 64]. Nowadays, many cytokines are known to share this property with IL-1, such as IL-2, IL-3, IL-6, IL-8, IL-11, IL-12, TNF and GM-CSF [64]. Thus, immune-derived products, such as cytokines, regulate neuroendocrine mechanisms, on one hand; while hormones, neurotransmitters, and neuropeptides modulate the outcome of a specific immune response, on the other [65, 66]. As a consequence of such a strong connection between these two systems, deregulation of the HPA axis or impairments in GCs actions affecting their availability and function are associated to the development of enhanced susceptibility to infection and inflammatory or autoimmune diseases [67–69].

The importance of GR in regulating the immune response has already been highlighted. GCs have been used as therapeutic drugs for autoimmune, inflammatory and allergic diseases for many years now in spite of their side effects. GCs side effects are generally associated to GR transactivation mechanism, since induction of bone, glucose and lipid metabolism-related genes relies on GR ability to positively regulate gene expression [5, 49]. Novel approaches to replace GCs in the treatment of such diseases involve the search for the so-called dissociated GR ligands [11, 70, 71]. As dissociated ligands, they are expected to possess the anti-inflammatory effects of classic GCs with no effect on bone, glucose or lipid metabolism. In this context, non-steroidal dissociated GR ligands are actively sought after, with preferential binding to GR against other steroid receptors and no interference with the steroidogenic pathway and steroid clearance that regulate GCs actions in target tissues [71]. Some of these non-steroidal GR ligands have proven to be effective in the treatment of autoimmune diseases such as collagen-induced arthritis, experimental autoimmune encephalomyelitis and experimental autoimmune neuritis [71].

GCs secreted to the periphery by the adrenal gland also signal back to the paraventricular nucleus (PVN) of the hypothalamus, the anterior pituitary and also the hippocampus, to negatively regulate the activation of the HPA axis in a GR-dependent manner. Pituitary corticotropes and PVN represent the primary feedback sites for endogenous GCs released during stress. However, the effect of GCs on information processing at the hippocampus may also result in adaptation of behavioral patterns, affecting the state of the HPA axis [72]. In this way, GCs decrease their own circulating levels through down-regulation of the HPA axis activation. Ultimately, this GR-mediated negative feedback loop maintains GCs levels under normal physiological conditions [73]. Interestingly, GR abnormal function appears to play a key role in pathophysiological conditions related to the HPA axis activity. Due to impaired

GR activity, GCs fail to exert negative feedback at the HPA axis. Consequently, GCs circulating levels remain high after stress and homeostasis is compromised. Such deregulation of the HPA axis has been associated to psychiatric disorders, and in particular major depression. Most depressed patients usually exhibit hyperactivity of the HPA axis and hypercholesterolemia [1, 73, 74].

Taking these considerations into account, GR malfunction has obvious functional consequences regarding both immune and neuroendocrine responses. In this context, the study of the molecular mechanisms implicated in the modulation of GR activity emerges as a field of utmost importance.

POST-TRANSLATIONAL MODIFICATIONS OF THE GLUCOCORTICOID RECEPTOR: IMMUNE AND NEUROENDOCRINE IMPLICATIONS

Given the fact that GR is ubiquitously expressed, different mechanisms have arisen to ensure specific responses to GCs depending on the target tissue. GCs secreted in response to stress reach different target organs, allowing the synchronization of body functions to efficiently develop stress-coping responses, including recovery and adaptation. Differences in ligand bioavailability, GR expression levels and cofactor availability contribute to GCs tissue-specific effects [75]. As previously stated, chromatin accessibility of GR to its responsive genes also appears to be critical for cell-specific outcome following exposure to GCs [33, 34]. However, it has recently gained interest the occurrence of GR protein isoforms and its post-translational modifications (PTMs) as crucial players in GR signaling upon ligand binding.

GR heterogeneity at the transcriptional and post-transcriptional levels arises from the existence of alternative splicing variants and alternative translation initiation sites, respectively [76]. Polymorphisms at the sequence level with an impact on GR activity have also been described, and could explain, in part, individual differences in health, disease and GCs treatment [77].

PTMs play a critical role in regulating protein stability, structure, function, intracellular localization and protein – protein interactions determining the final outcome of cellular processes. In particular, GR has been reported to be target of many PTMs that include phosphorylation, acetylation, ubiquitination and small ubiquitin-like modifier (SUMO) conjugation.

Phosphorylation of GR has been shown to be critical for its activation. Many target phosphorylation residues have been characterized up to date-murine GR bears eight phosphorylation sites, while only five experimentally confirmed phosphorylation sites were reported on human GR-, mainly positioned in the N-terminal domain. Phosphorylation at each residue has a specific effect on GR activity that can be either positive or negative [7]. It was demonstrated that GR is phosphorylated in a hormone-dependent manner [78] and that differentially phosphorylated GR species show specific intracellular sub-localization [79]. Intriguingly, specific GR phosphorylated forms were differentially recruited to promoters of target genes and selectively regulated their expression. In addition, phosphorylation status of individual residues seems to have different impact depending on the target gene under analysis [80, 81]. Recently, it was described that GR phosphorylation occurs not only in a hormone-dependent manner but also, previous to hormone binding, as a consequence of cellular stress, therefore regulating GR response upon ligand stimulation. This newly described mechanism suggests that cellular history prior to GCs signaling, measured by phosphorylation of the GR, has an impact on the regulation of its target genes [82]. In addition, GR protein stability has been shown to be dependent on its phosphorylation state, since phosphorylation mutants displayed increased protein stability and decreased sensitivity to ligand-induced reduction in protein levels [83]. Therefore, phosphorylation regulates crucial aspects of GCs signaling through its functional impact on GR activity. In this regard, phosphorylation of GR was shown to be altered due to stress. In a chronic mild stress model of depression, GR phosphorylation was reduced in the prefrontal cortex together with the expression of GR target genes, suggesting one plausible explanation for this latter observation. In this same work, antidepressant treatment decreased GR phosphorylation observed in stressed rats compared to control, rendering GR phosphorylation a putative target for antidepressant actions [84]. In line with these results, it was demonstrated that GR phosphorylation patterns as well as hippocampal neurogenesis were altered by treatment with antidepressants in a hippocampal progenitor cell line [85]. Interestingly, during inflammation, stimulation through pro-inflammatory signals culminates in the activation of AP-1 and NF-kB, together with other relevant inflammatory TFs, which in turn induce the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules, to propagate cellular inflammation. Activation of such inflammatory

pathways involves the participation of kinases as mediators. Remarkably, these same kinases (like JNK, p38 and extracellular-signal regulated kinases -ERK-) are involved in modulation of GR activity through phosphorylation. At the same time, GR regulates activation of these pathways by modulating the activity of the kinases, thus contributing to the complexity of the landscape [7]. Moreover, chaperones complexes and coregulators are also targeted by phosphorylation, which alters their functions and thus impacts on GR activity [86–88].

Like other nuclear receptors belonging to the same superfamily such as the estrogen and androgen receptors, the GR is acetylated in lysine residues within its DNA binding domain. Deacetylation of GR by HDAC2 was found to be necessary for GR transrepression of NF-kB, suggesting that GR deacetylation may be a prerequisite for its repressive action on NF-kB-mediated gene expression [89]. In particular, HDAC2 levels were found to be critical for GCs response in patients suffering from a chronic inflammatory disease, chronic obstructive pulmonary disease. Primary alveolar macrophages from these patients, insensitive to GCs anti-inflammatory actions, presented low HDAC2 protein levels. Consistently, over-expression of HDAC2 in these cultures restored GCs sensitivity [89], pointing to a key role for HDAC2 and GR acetylation in the regulation of inflammatory immune responses. However, acetylation/deacetylation of GR was found to be relevant not only for transrepression but also for transactivation, since the HAT protein CLOCK repressed GR transcriptional activity by acetylating GR target lysine residues [90]. Together with GR, other proteins directly or indirectly regulating GR activity are modified by acetylation. In this context, the most relevant target of acetylation is Hsp90. It was demonstrated that Hsp90 acetylation regulates its interaction with client proteins, including co-chaperones such as p23 and FKBP52 [91], and its deacetylation by HDAC6 is critical for GR complex maturation, since HDAC6-deficient cells showed defective GR ligand binding, nuclear translocation and transcriptional activation [92]. In line with these results, the lack of HDAC6 results in deregulation of GR-Hsp90 complex assembly/disassembly and thus GR activity [93]. Finally, histone acetylation also plays a critical role in GR-mediated regulation of responsive genes. As previously described, histone acetylation/deacetylation is crucial for both GR-mediated transactivation and transrepression.

The ubiquitin/proteasome pathway has also been shown to play a critical role in GR activity. In fact, GR was found to be a ubiquitin target itself. Proteasome inhibition leads to decreased ligand-induced GR protein down-regulation and enhanced GR transcriptional activity [94, 95]. In support of these findings, mutation of the ubiquitin-target lysine within the PEST motif – these motifs are associated to protein degradation by proteasome [96] – mimics the effect of proteasome inhibition, rendering GR protein levels independent of ligand-induced degradation and enhancing GR transcriptional activity as well [94, 97]. In addition, proteasome inhibition alters GR nuclear trafficking together with GR binding to the nuclear matrix [95]. Interestingly, inhibition of proteasome activity affects GR-target gene expression not only by altering GR proteasomal degradation but also by modulating histone methylation and RNA polymerase II association with chromatin as it was shown at the MMTV activated gene [98]. Therefore, a link between chromatin structure and proteasome activity at GR target genes arises as a plausible explanation for proteasome regulation of GR activity, beyond proteasome-mediated GR degradation [98, 99]. Proteasome components are also found at the MMTV promoter regulating rapid GR exchange at this site, pointing to the proteasome as a regulator of hormone sensing and fine-tuning of GR responses to variable conditions [100].

However, proteasome-mediated degradation of GR appears to be a cell-type-dependent regulatory mechanism. In this respect, conflicting results have been obtained from *in vitro* and *in vivo* studies in maturing and developing neurons regarding the effects of GCs on GR protein levels [101–103]. Under chronic GCs exposure, GR protein levels are not altered in a hippocampal context by hormone-induced down-regulation [102]. Proteasome dependence is recovered when over-expressing the C-terminus of Hsc70-interacting protein (CHIP), an E3 ubiquitin ligase, which in turn alters GR transactivation activity [104, 105]. Therefore, these results suggest that relative abundance of an E3 ligase might confer differential GR sensitivity in a neuronal context. Interestingly, CHIP is a component of the Hsp90 hetero-complex [104] and also targets Hsp90 for proteasomal degradation [106–108], consequently regulating GR activity [104].

Together with GR, nuclear receptor coregulators are also subjected to regulation by the ubiquitin/proteasome pathway [109]. Interestingly, components of the ubiquitin/proteasome pathway have also been described to act as nuclear receptor coregulators themselves, thereby providing a new link between nuclear receptors and the proteasome/ubiquitin pathway [99].

Tightly linked to ubiquitination, SUMO conjugation has also been found to play an important role in modulating GR transcriptional activity. SUMO conjugation is an enzymatic reversible reaction that involves the covalently addition of a SUMO peptide to a variety of target proteins [110]. Substrate modification by SUMO attachment has a wide range of consequences that include alterations in protein-protein interaction, protein intracellular localization and protein activity [111]. GR SUMO conjugation was originally described more than a decade ago [112, 113], when GR was identified as a SUMO target. A quantitative proteomic analysis identified later GR as a SUMO target in cells subjected to heat-shock stress [114], pointing to a possible role for GR SUMOylation in the modulation of its transcriptional activity under cellular stress conditions. GR bears three SUMOylation target sites; two of them reside in the N-terminal domain within a so-called synergy control (SC) motif that is critical for GR transactivation while the third one lies within the LBD [115, 116]. SC motifs limit transcriptional synergy of multiple DNA-bound regulators at compound binding sites [117]. SUMO conjugation at these sites in the GR N-terminal domain enhances the ability of the SC motif to repress transcriptional activation, pointing at SUMOylation as a regulatory mechanism to control the output of GR transcriptional activation and supporting a direct inhibitory function for SUMO in this context [115, 116]. Based on these results, it has been hypothesized that GR SUMOylation within its SC motif would affect recruitment of corepressors and therefore transcriptional regulation of down-stream genes [117, 118]. We have demonstrated that the presence of a SUMOylation enhancer, RSUME [119], uncovers a positive role for SUMO conjugation to the LBD acceptor site in GR-mediated transcriptional regulation [120]. Moreover, SUMOylation at this residue appears to be critical for cofactor-mediated GR activity, since its point mutation diminishes GRIP1 coactivator activity in transactivation assays while it does not disrupt GR-GRIP1 interaction [120]. Interestingly, GRIP1, as many other coregulators, is also subjected to SUMO modification, which finally influences its nuclear receptor coactivation activity [121].

A genome-wide analysis of GR SUMOylation impact on gene expression revealed that both hormone up- and down-regulated genes are affected by SUMO modification of the GR. Remarkably, genes differentially regulated by GR SUMOylation are significantly related to proliferation and apoptosis pathways. In addition, GR chromatin occupancy is also dependent on GR SUMO modification in a target locus-selective fashion [122].

Other components of the SUMO pathway also play an important role in regulating GR activity. The E2-conjugating enzyme of the SUMO pathway Ubc9 binds to the GR [123] and modulates GR-mediated transcription independently of its SUMOylation activity [124, 125]. Members of the protein inhibitor of activated STAT (PIAS) family, known to be SUMO E3 ligases, regulate GR-mediated transcription as well, acting both as coactivators and corepressors in a promoter context-dependent manner [126]. In particular, some PIAS family members were shown to alter GR activity in a neuroblastoma cell line [127].

The relevance of SUMO conjugation in neuroendocrine and immune functions is currently under thorough study, although its importance regarding GR signaling pathway in these contexts is yet to be uncovered. Since SUMOylation factors like RSUME are induced under stress [119, 120], they might contribute to fine-tune GR responses during stress adaptation.

CONCLUDING REMARKS

The neuroendocrine and immune systems are tightly connected through multiple pathways that orchestrate concerted responses. GCs, as the most downstream effectors of the HPA axis, play a critical role in the communication between these systems and preservation of their homeostatic balance (Fig. 1). As a consequence, abnormal GCs signaling results in the onset of neuroendocrine and immune pathological conditions that have mutual influence and modulate the response of one another. Since GCs effects are mainly mediated by GR, the development of therapeutic strategies to overcome these conditions necessarily requires the comprehensive understanding of the molecular mechanisms underlying GR biological actions. In this regard, PTMs arise as critical modulators of GR activity. GR is subjected to PTMs that fine-tune its activity under certain circumstances (Fig. 1). Together with GR, other proteins such as chaperones and coregulators involved in GR-mediated responses are also targets of PTMs and contribute to the final outcome of GCs signaling. However, the relevance of these PTMs on GR and proteins affecting GR activity should be carefully analyzed taking into consideration the cellular context. The occurrence of these PTMs contributes to the development of tissue-specific responses. Therefore, the study of the effect of PTMs in the neuroendocrine and immune contexts is vital to the development

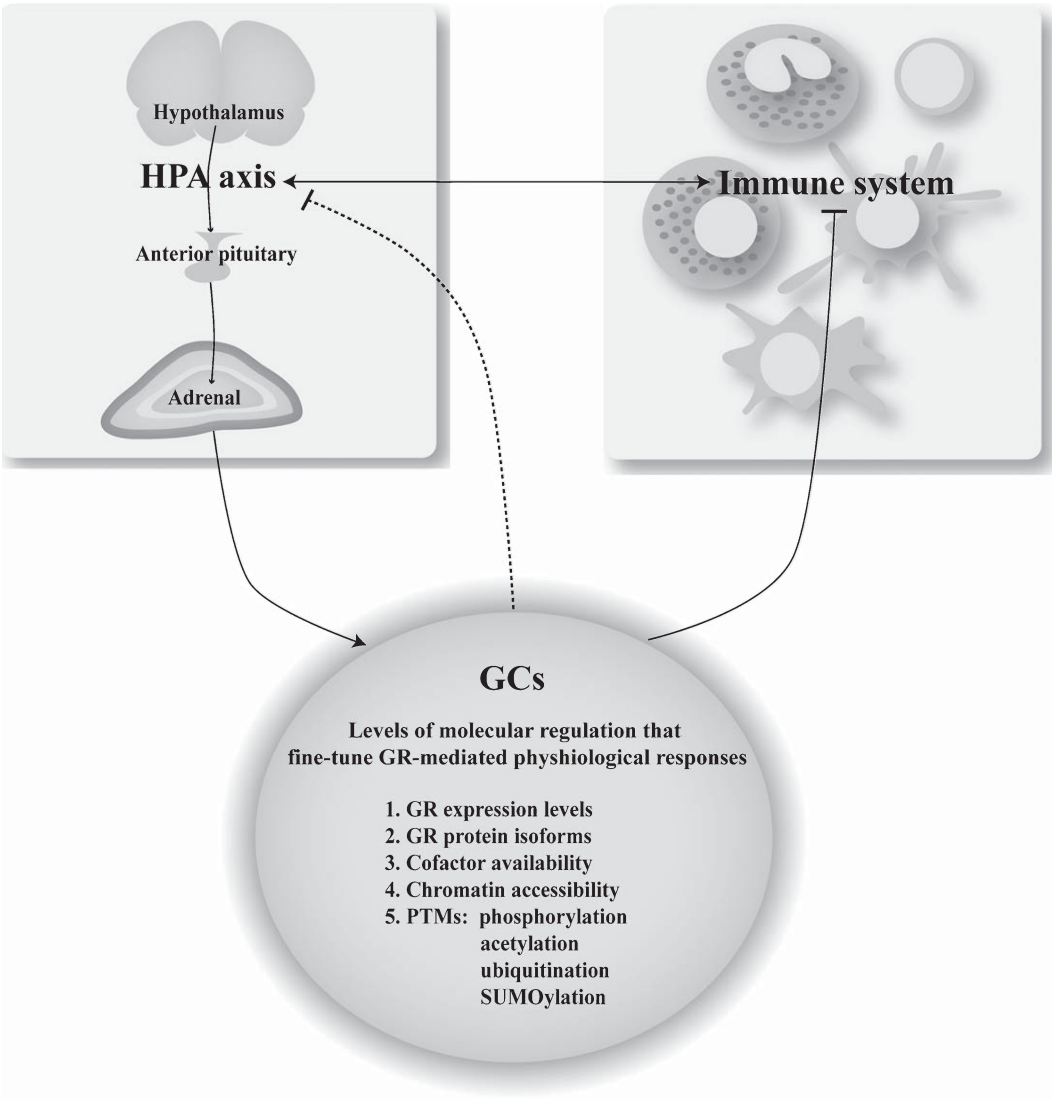

Fig. 1. Homeostatic balance of the neuroendocrine and immune systems. GCs, as the most downstream effectors of the HPA axis, are key mediators in the communication between the neuroendocrine and immune systems. GCs impact on both HPA-mediated stress responses and immune responses. They exert their effects mainly through binding to the GR. Giving their wide range of actions, a series of molecular mechanisms regulate GR activity to ensure GCs specificity and potency. These mechanisms include modulation of GR expression levels, GR protein isoforms, cofactor availability, chromatin accessibility and PTMs. As such, PTMs play a crucial role in the regulation of GR activity and comprise phosphorylation, acetylation, ubiquitination and SUMOylation.

of therapeutic approaches aimed at modulating GR activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES

[1] de Kloet ER, Joels M, Holsboer F. Stress and the brain: From adaptation to disease. Nat Rev Neurosci. 2005; 6(6): 463-475. doi: 10.1038/nrn1683

[2] Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev. 2000; 21(1): 55-89. doi: 10.1210/er.21.1.55

[3] Liberman AC, Druker J, Perone MJ, Arzt E. Glucocorticoids in the regulation of transcription factors that control cytokine synthesis. Cytokine Growth Factor Rev. 2007; 18(1-2): 45-56. doi: 10.1016/j.cytogfr.2007.01.005

[4] Boumpas DT, Chrousos GP, Wilder RL, Cupps TR, Balow JE. Glucocorticoid therapy for immune-mediated diseases: Basic and clinical correlates. Ann Intern Med. 1993; 119(12): 1198-1208.

[5] Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther. 2002; 96(1): 23-43. doi: 10.1016/S0163-7258(02)00297-8

[6] Evans RM. The steroid and thyroid hormone receptor superfamily. Science. 1988; 240(4854): 889-895. doi: 10.1126/science.3283939

[7] Beck IM, Vanden Berghe W, Vermeulen L, Yamamoto KR, Haegeman G, De Bosscher K. Crosstalk in inflammation: The interplay of glucocorticoid receptor-based mechanisms and kinases and phosphatases. Endocr Rev. 2009; 30(7): 830-882. doi: 10.1210/er.2009-0013

[8] Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, McKee DD, et al. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. Cell. 2002; 110(1): 93-105. doi: 10.1016/S0092-8674(02)00817-6

[9] Lee GS, Simons SS, Jr. Ligand binding domain mutations of the glucocorticoid receptor selectively modify the effects with, but not binding of, cofactors. Biochemistry. 2011; 50(3): 356-366. doi: 10.1021/bi101792d

[10] Pratt WB, Toft DO. Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev. 1997; 18(3): 306-360. doi: 10.1210/er.18.3.306

[11] De Bosscher K, Haegeman G. Minireview: Latest perspectives on antiinflammatory actions of glucocorticoids. Mol Endocrinol. 2009; 23(3): 281-291. doi: 10.1210/me.2008-0283

[12] Madan AP, DeFranco DB. Bidirectional transport of glucocorticoid receptors across the nuclear envelope. Proc Natl Acad Sci U S A. 1993; 90(8): 3588-3592. doi: 10.1073/pnas.90.8.3588

[13] Elbi C, Walker DA, Romero G, Sullivan WP, Toft DO, Hager GL, et al. Molecular chaperones function as steroid receptor nuclear mobility factors. Proc Natl Acad Sci U S A. 2004; 101(9): 2876-2881. doi: 10.1073/pnas.0400116101

[14] Vandevyver S, Dejager L, Libert C. On the trail of the glucocorticoid receptor: Into the nucleus and back. Traffic. 2012; 13(3): 364-374. doi: 10.1111/j.1600-0854.2011.01288.x

[15] Denis M, Gustafsson JA. The Mr approximately 90,000 heat shock protein: An important modulator of ligand and DNA-binding properties of the glucocorticoid receptor. Cancer Res. 1989; 49(8 Suppl): 2275s-2281s.

[16] Picard D. Chaperoning steroid hormone action. Trends Endocrinol Metab. 2006; 17(6): 229-235. doi: 10.1016/j.tem.2006.06.003

[17] Sanchez ER. Chaperoning steroidal physiology: Lessons from mouse genetic models of Hsp90 and its cochaperones. Biochim Biophys Acta. 2012; 1823(3): 722-729. doi: 10.1016/j.bbamcr.2011.11.006

[18] Kovacs JJ, Cohen TJ, Yao TP. Chaperoning steroid hormone signaling via reversible acetylation. Nucl Recept Signal. 2005; 3: e004. doi: 10.1621/nrs.03004

[19] Bucci M, Roviezzo F, Cicala C, Sessa WC, Cirino G. Geldanamycin, an inhibitor of heat shock protein 90 (Hsp90) mediated signal transduction has anti-inflammatory effects and interacts with glucocorticoid receptor *in vivo*. Br J Pharmacol. 2000; 131(1): 13-16. doi: 10.1038/sj.bjp.0703549

[20] Tago K, Tsukahara F, Naruse M, Yoshioka T, Takano K. Hsp90 inhibitors attenuate effect of dexamethasone on activated NF-kappaB and AP-1. Life Sci. 2004; 74(16): 1981-1992. doi: 10.1016/j.lfs.2003.07.056

[21] Rosenhagen MC, Soti C, Schmidt U, Wochnik GM, Hartl FU, Holsboer F, et al. The heat shock protein 90-targeting drug cisplatin selectively inhibits steroid receptor activation. Mol Endocrinol. 2003; 17(10): 1991-2001. doi: 10.1210/me.2003-0141

[22] Galigniana MD, Harrell JM, Housley PR, Patterson C, Fisher SK, Pratt WB. Retrograde transport of the glucocorticoid receptor in neurites requires dynamic assembly of complexes with the protein chaperone hsp90 and is linked to the CHIP component of the machinery for proteasomal degradation. Brain Res Mol Brain Res. 2004; 123(1-2): 27-36. doi: 10.1016/j.molbrainres.2003.12.015

[23] Noguchi T, Makino S, Matsumoto R, Nakayama S, Nishiyama M, Terada Y, et al. Regulation of glucocorticoid receptor transcription and nuclear translocation during single and repeated immobilization stress. Endocrinology. 2010; 151(9): 4344-4355. doi: 10.1210/en.2010-0266

[24] Shen HY, Zhao Y, Chen XY, Xiong RP, Lu JL, Chen JF, et al. Differential alteration of heat shock protein 90 in mice modifies glucocorticoid receptor function and susceptibility to trauma. J Neurotrauma. 2010; 27(2): 373-381. doi: 10.1089/neu.2009.0926

[25] Espallergues J, Teegarden SL, Veerakumar A, Boulden J, Challis C, Jochems J, et al. HDAC6 regulates glucocorticoid receptor signaling in serotonin pathways with critical impact on stress resilience. J Neurosci. 2012; 32(13): 4400-4416. doi: 10.1523/JNEUROSCI.5634-11.2012

[26] Wochnik GM, Ruegg J, Abel GA, Schmidt U, Holsboer F, Rein T. FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. J Biol Chem. 2005; 280(6): 4609-4616. doi: 10.1074/jbc.M407498200

[27] Davies TH, Ning YM, Sanchez ER. A new first step in activation of steroid receptors: Hormone-induced switching of FKBP51 and FKBP52 immunophilins. J Biol Chem. 2002; 277(7): 4597-4600. doi: 10.1074/jbc.C100531200

[28] Paakinaho V, Makkonen H, Jaaskelainen T, Palvimo JJ. Glucocorticoid receptor activates poised FKBP51 locus through long-distance interactions. Mol Endocrinol. 2010; 24(3): 511-525. doi: 10.1210/me.2009-0443

[29] Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology. 2009; 34 Suppl 1: S186-S195. doi: 10.1016/j.psyneuen.2009.05.021

[30] Storer CL, Dickey CA, Galigniana MD, Rein T, Cox MB. FKBP51 and FKBP52 in signaling and disease. Trends Endocrinol Metab. 2011; 22(12): 481-490. doi: 10.1016/j.tem.2011.08.001

[31] Sivils JC, Storer CL, Galigniana MD, Cox MB. Regulation of steroid hormone receptor function by the 52-kDa FK506-binding protein (FKBP52). Curr Opin Pharmacol. 2011; 11(4): 314-319. doi: 10.1016/j.coph.2011.03.010

[32] Hartmann J, Wagner KV, Dedic N, Marinescu D, Scharf SH, Wang XD, et al. Fkbp52 heterozygosity alters behavioral, endocrine and neurogenetic parameters under basal

and chronic stress conditions in mice. Psychoneuroendocrinology. 2012; 37(12): 2009-2021. doi: 10.1016/j.psyneuen.2012.04.017

[33] John S, Sabo PJ, Johnson TA, Sung MH, Biddie SC, Lightman SL, et al. Interaction of the glucocorticoid receptor with the chromatin landscape. Mol Cell. 2008; 29(5): 611-624. doi: 10.1016/j.molcel.2008.02.010

[34] John S, Sabo PJ, Thurman RE, Sung MH, Biddie SC, Johnson TA, et al. Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. Nat Genet. 2011; 43(3): 264-268. doi: 10.1038/ng.759

[35] De Bosscher K, Vanden Berghe W, Haegeman G. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: Molecular mechanisms for gene repression. Endocr Rev. 2003; 24(4): 488-522. doi: 10.1210/er.2002-0006

[36] Liberman AC, Refojo D, Druker J, Toscano M, Rein T, Holsboer F, et al. The activated glucocorticoid receptor inhibits the transcription factor T-bet by direct protein-protein interaction. FASEB J. 2007; 21(4): 1177-1188. doi: 10.1096/fj.06-7452com

[37] Ratman D, Vanden Berghe W, Dejager L, Libert C, Tavernier J, Beck IM, et al. How glucocorticoid receptors modulate the activity of other transcription factors: A scope beyond tethering. Mol Cell Endocrinol. 2013; 380(1-2): 41-54. doi: 10.1016/j.mce.2012.12.014

[38] Surjit M, Ganti KP, Mukherji A, Ye T, Hua G, Metzger D, et al. Widespread negative response elements mediate direct repression by agonist-liganded glucocorticoid receptor. Cell. 2011; 145(2): 224-241. doi: 10.1016/j.cell.2011.03.027

[39] Hudson WH, Youn C, Ortlund EA. The structural basis of direct glucocorticoid-mediated transrepression. Nat Struct Mol Biol. 2013; 20(1): 53-58. doi: 10.1038/nsmb.2456

[40] Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev. 2000; 14(2): 121-141. doi: 10.1101/gad.14.2.121

[41] McKenna NJ, O'Malley BW. Combinatorial control of gene expression by nuclear receptors and coregulators. Cell. 2002; 108(4): 465-474. doi: 10.1016/S0092-8674(02)00641-4

[42] Wang Q, Blackford JA Jr, Song LN, Huang Y, Cho S, Simons SS Jr. Equilibrium interactions of corepressors and coactivators with agonist and antagonist complexes of glucocorticoid receptors. Mol Endocrinol. 2004; 18(6): 1376-1395. doi: 10.1210/me.2003-0421

[43] Kroe RR, Baker MA, Brown MP, Farrow NA, Gautschi E, Hopkins JL, et al. Agonist versus antagonist induce distinct thermodynamic modes of co-factor binding to the glucocorticoid receptor. Biophys Chem. 2007; 128(2-3): 156-164. doi: 10.1016/j.bpc.2007.03.013

[44] Ronacher K, Hadley K, Avenant C, Stubsrud E, Simons SS Jr, Louw A, et al. Ligand-selective transactivation and transrepression via the glucocorticoid receptor: Role of cofactor interaction. Mol Cell Endocrinol. 2009; 299(2): 219-231. doi: 10.1016/j.mce.2008.10.008

[45] Lonard DM, O'Malley BW. Expanding functional diversity of the coactivators. Trends Biochem Sci. 2005; 30(3): 126-132. doi: 10.1016/j.tibs.2005.01.001

[46] Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, et al. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell. 1996; 85(3): 403-414. doi: 10.1016/S0092-8674(00)81118-6

[47] De Bosscher K, Vanden Berghe W, Haegeman G. Glucocorticoid repression of AP-1 is not mediated by competition for nuclear coactivators. Mol Endocrinol. 2001; 15(2): 219-227. doi: 10.1210/me.15.2.219

[48] De Bosscher K, Vanden Berghe W, Vermeulen L, Plaisance S, Boone E, Haegeman G. Glucocorticoids repress NF-kappaB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. Proc Natl Acad Sci U S A. 2000; 97(8): 3919-3924. doi: 10.1073/pnas.97.8.3919

[49] Glass CK, Saijo K. Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. Nat Rev Immunol. 2010; 10(5): 365-376. doi: 10.1038/nri2748

[50] Schulz M, Eggert M, Baniahmad A, Dostert A, Heinzel T, Renkawitz R. RU486-induced glucocorticoid receptor agonism is controlled by the receptor N terminus and by corepressor binding. J Biol Chem. 2002; 277(29): 26238-26243. doi: 10.1074/jbc.M203268200

[51] Rosenfeld MG, Lunyak VV, Glass CK. Sensors and signals: A coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. Genes Dev. 2006; 20(11): 1405-1428. doi: 10.1101/gad.1424806

[52] Rogatsky I, Luecke HF, Leitman DC, Yamamoto KR. Alternate surfaces of transcriptional coregulator GRIP1 function in different glucocorticoid receptor activation and repression contexts. Proc Natl Acad Sci U S A. 2002; 99(26): 16701-16706. doi: 10.1073/pnas.262671599

[53] Uhlenhaut NH, Barish GD, Yu RT, Downes M, Karunasiri M, Liddle C, et al. Insights into negative regulation by the glucocorticoid receptor from genome-wide profiling of inflammatory cistromes. Mol Cell. 2013; 49(1): 158-171. doi: 10.1016/j.molcel.2012.10.013

[54] Urnov FD, Wolffe AP. Chromatin remodeling and transcriptional activation: The cast (in order of appearance). Oncogene. 2001; 20(24): 2991-3006. doi: 10.1038/sj.onc.1204323

[55] Ito K, Lim S, Caramori G, Cosio B, Chung KF, Adcock IM, et al. A molecular mechanism of action of theophylline: Induction of histone deacetylase activity to decrease inflammatory gene expression. Proc Natl Acad Sci U S A. 2002; 99(13): 8921-8926. doi: 10.1073/pnas.132556899

[56] Bilodeau S, Vallette-Kasic S, Gauthier Y, Figarella-Branger D, Brue T, Berthelet F, et al. Role of Brg1 and HDAC2 in GR trans-repression of the pituitary POMC gene and misexpression in Cushing disease. Genes Dev. 2006; 20(20): 2871-2886. doi: 10.1101/gad.1444606

[57] Ramamoorthy S, Cidlowski JA. Ligand-induced repression of the glucocorticoid receptor gene is mediated by an NCoR1 repression complex formed by long-range chromatin interactions with intragenic glucocorticoid response elements. Mol Cell Biol. 2013; 33(9): 1711-1722. doi: 10.1128/MCB.01151-12

[58] Bresnick EH, John S, Berard DS, LeFebvre P, Hager GL. Glucocorticoid receptor-dependent disruption of a specific nucleosome on the mouse mammary tumor virus promoter is prevented by sodium butyrate. Proc Natl Acad Sci U S A. 1990; 87(10): 3977-3981. doi: 10.1073/pnas.87.10.3977

[59] Qiu Y, Zhao Y, Becker M, John S, Parekh BS, Huang S, et al. HDAC1 acetylation is linked to progressive modulation of steroid receptor-induced gene transcription. Mol Cell. 2006; 22(5): 669-679. doi: 10.1016/j.molcel.2006.04.019

[60] Luo Y, Jian W, Stavreva D, Fu X, Hager G, Bungert J, et al. Trans-regulation of histone deacetylase activities through acetylation. J Biol Chem. 2009; 284(50): 34901-34910. doi: 10.1074/jbc.M109.038356

[61] Kadiyala V, Patrick NM, Mathieu W, Jaime-Frias R, Pookhao N, An L, et al. Class I lysine deacetylases facilitate glucocorticoid-induced transcription. J Biol Chem. 2013; 288(40): 28900-28912. doi: 10.1074/jbc.M113.505115

[62] Marques-Deak A, Cizza G, Sternberg E. Brain-immune interactions and disease susceptibility. Mol Psychiatry. 2005; 10(3): 239-250. doi: 10.1038/sj.mp.4001643

[63] Besedovsky H, del Rey A, Sorkin E, Dinarello CA. Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. Science. 1986; 233(4764): 652-654. doi: 10.1126/science.3014662

[64] Besedovsky HO, Rey AD. Physiology of psychoneuroimmunology: A personal view. Brain Behav Immun. 2007; 21(1): 34-44. doi: 10.1016/j.bbi.2006.09.008

[65] Arzt E, Pereda MP, Castro CP, Pagotto U, Renner U, Stalla GK. Pathophysiological role of the cytokine network in the anterior pituitary gland. Front Neuroendocrinol. 1999; 20(1): 71-95. doi: 10.1006/frne.1998.0176

[66] Besedovsky HO, del Rey A. Immune-neuro-endocrine interactions: Facts and hypotheses. Endocr Rev. 1996; 17(1): 64-102.

[67] Webster JI, Tonelli L, Sternberg EM. Neuroendocrine regulation of immunity. Annu Rev Immunol. 2002; 20: 125-163. doi: 10.1146/annurev.immunol.20.082401.104914

[68] Charmandari E, Tsigos C, Chrousos G. Endocrinology of the stress response. Annu Rev Physiol. 2005; 67: 259-284. doi: 10.1146/annurev.physiol.67.040403.120816

[69] Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: From HPA axis to glucocorticoid receptor dysfunction. Ann N Y Acad Sci. 2012; 1261: 55-63. doi: 10.1111/j.1749-6632.2012.06633.x

[70] Newton R, Holden NS. Separating transrepression and transactivation: A distressing divorce for the glucocorticoid receptor? Mol Pharmacol. 2007; 72(4): 799-809. doi: 10.1124/mol.107.038794

[71] De Bosscher K, Beck IM, Haegeman G. Classic glucocorticoids versus non-steroidal glucocorticoid receptor modulators: Survival of the fittest regulator of the immune system? Brain Behav Immun. 2010; 24(7): 1035-1042. doi: 10.1016/j.bbi.2010.06.010

[72] De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. Brain corticosteroid receptor balance in health and disease. Endocr Rev. 1998; 19(3): 269-301. doi: 10.1210/er.19.3.269

[73] Anacker C, Zunszain PA, Carvalho LA, Pariante CM. The glucocorticoid receptor: Pivot of depression and of antidepressant treatment? Psychoneuroendocrinology. 2011; 36(3): 415-425. doi: 10.1016/j.psyneuen.2010.03.007

[74] Pariante CM, Lightman SL. The HPA axis in major depression: Classical theories and new developments. Trends Neurosci. 2008; 31(9): 464-468. doi: 10.1016/j.tins.2008.06.006

[75] Oakley RH, Cidlowski JA. Cellular processing of the glucocorticoid receptor gene and protein: New mechanisms for generating tissue-specific actions of glucocorticoids. J Biol Chem. 2011; 286(5): 3177-3184. doi: 10.1074/jbc.R110.179325

[76] Kino T, Su YA, Chrousos GP. Human glucocorticoid receptor isoform beta: Recent understanding of its potential implications in physiology and pathophysiology. Cell Mol Life Sci. 2009; 66(21): 3435-3448. doi: 10.1007/s00018-009-0098-z

[77] Gross KL, Cidlowski JA. Tissue-specific glucocorticoid action: A family affair. Trends Endocrinol Metab. 2008; 19(9): 331-339. doi: 10.1016/j.tem.2008.07.009

[78] Orti E, Mendel DB, Smith LI, Munck A. Agonist-dependent phosphorylation and nuclear dephosphorylation of glucocorticoid receptors in intact cells. J Biol Chem. 1989; 264(17): 9728-9731.

[79] Wang Z, Frederick J, Garabedian MJ. Deciphering the phosphorylation "code" of the glucocorticoid receptor *in vivo*. J Biol Chem. 2002; 277(29): 26573-26580. doi: 10.1074/jbc.M110530200

[80] Wang Z, Chen W, Kono E, Dang T, Garabedian MJ. Modulation of glucocorticoid receptor phosphorylation and transcriptional activity by a C-terminal-associated protein phosphatase. Mol Endocrinol. 2007; 21(3): 625-634. doi: 10.1210/me.2005-0338

[81] Blind RD, Garabedian MJ. Differential recruitment of glucocorticoid receptor phospho-isoforms to glucocorticoid-induced genes. J Steroid Biochem Mol Biol. 2008; 109(1-2): 150-157. doi: 10.1016/j.jsbmb.2008.01.002

[82] Galliher-Beckley AJ, Williams JG, Cidlowski JA. Ligand-independent phosphorylation of the glucocorticoid receptor integrates cellular stress pathways with nuclear receptor signaling. Mol Cell Biol. 2011; 31(23): 4663-4675. doi: 10.1128/MCB.05866-11

[83] Webster JC, Jewell CM, Bodwell JE, Munck A, Sar M, Cidlowski JA. Mouse glucocorticoid receptor phosphorylation status influences multiple functions of the receptor protein. J Biol Chem. 1997; 272(14): 9287-9293. doi: 10.1074/jbc.272.14.92

[84] Guidotti G, Calabrese F, Anacker C, Racagni G, Pariante CM, Riva MA. Glucocorticoid receptor and FKBP5 expression is altered following exposure to chronic stress: Modulation by antidepressant treatment. Neuropsychopharmacology. 2013; 38(4): 616-627. doi: 10.1038/npp.2012.225

[85] Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S, et al. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Mol Psychiatry. 2011; 16(7): 738-750. doi: 10.1038/mp.2011.26

[86] Weigel NL, Moore NL. Kinases and protein phosphorylation as regulators of steroid hormone action. Nucl Recept Signal. 2007; 5: e005. doi: 10.1621/nrs.05005

[87] Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer. 2010; 10(8): 537-549. doi: 10.1038/nrc2887

[88] Trevino LS, Weigel NL. Phosphorylation: A fundamental regulator of steroid receptor action. Trends Endocrinol Metab. 2013; 24(10): 515-524. doi: 10.1016/j.tem.2013.05.008

[89] Ito K, Yamamura S, Essilfie-Quaye S, Cosio B, Ito M, Barnes PJ, et al. Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-kappaB suppression. J Exp Med. 2006; 203(1): 7-13. doi: 10.1084/jem.20050466

[90] Nader N, Chrousos GP, Kino T. Circadian rhythm transcription factor CLOCK regulates the transcriptional activity of the glucocorticoid receptor by acetylating its hinge region lysine cluster: Potential physiological implications. FASEB J. 2009; 23(5): 1572-1583. doi: 10.1096/fj.08-117697

[91] Scroggins BT, Robzyk K, Wang D, Marcu MG, Tsutsumi S, Beebe K, et al. An acetylation site in the middle domain of Hsp90 regulates chaperone function. Mol Cell. 2007; 25(1): 151-159. doi: 10.1016/j.molcel.2006.12.008

[92] Kovacs JJ, Murphy PJ, Gaillard S, Zhao X, Wu JT, Nicchitta CV, et al. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. Mol Cell. 2005; 18(5): 601-607. doi: 10.1016/j.molcel.2005.04.021

[93] Murphy PJ, Morishima Y, Kovacs JJ, Yao TP, Pratt WB. Regulation of the dynamics of hsp90 action on the glucocorticoid receptor by acetylation/deacetylation of the chaperone. J Biol Chem. 2005; 280(40): 33792-33799. doi: 10.1074/jbc.M506997200

[94] Wallace AD, Cidlowski JA. Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. J Biol Chem. 2001; 276(46): 42714-42721. doi: 10.1074/jbc.M106033200

[95] Deroo BJ, Rentsch C, Sampath S, Young J, DeFranco DB, Archer TK. Proteasomal inhibition enhances glucocorticoid receptor transactivation and alters its subnuclear trafficking. Mol Cell Biol. 2002; 22(12): 4113-4123. doi: 10.1128/MCB.22.12.4113-4123.2002

[96] Rechsteiner M, Rogers SW. PEST sequences and regulation by proteolysis. Trends Biochem Sci. 1996; 21(7): 267-271. doi: 10.1016/S0968-0004(96)10031-1

[97] Wallace AD, Cao Y, Chandramouleeswaran S, Cidlowski JA. Lysine 419 targets human glucocorticoid receptor for proteasomal degradation. Steroids. 2010; 75(12): 1016-1023. doi: 10.1016/j.steroids.2010.06.015

[98] Kinyamu HK, Archer TK. Proteasome activity modulates chromatin modifications and RNA polymerase II phosphorylation to enhance glucocorticoid receptor-mediated transcription. Mol Cell Biol. 2007; 27(13): 4891-4904. doi: 10.1128/MCB.02162-06

[99] Kinyamu HK, Chen J, Archer TK. Linking the ubiquitin-proteasome pathway to chromatin remodeling/modification by nuclear receptors. J Mol Endocrinol. 2005; 34(2): 281-297. doi: 10.1677/jme.1.01680

[100] Stavreva DA, Muller WG, Hager GL, Smith CL, McNally JG. Rapid glucocorticoid receptor exchange at a promoter is coupled to transcription and regulated by chaperones and proteasomes. Mol Cell Biol. 2004; 24(7): 2682-2697. doi: 10.1128/MCB.24.7.2682-2697.2004

[101] Sapolsky RM, McEwen BS. Down-regulation of neural corticosterone receptors by corticosterone and dexamethasone. Brain Res. 1985; 339(1): 161-165. doi: 10.1016/0006-8993(85)90638-9

[102] Wang X, Pongrac JL, DeFranco DB. Glucocorticoid receptors in hippocampal neurons that do not engage proteasomes escape from hormone-dependent down-regulation but maintain transactivation activity. Mol Endocrinol. 2002; 16(9): 1987-1998. doi: 10.1210/me.2001-0287

[103] Conway-Campbell BL, McKenna MA, Wiles CC, Atkinson HC, de Kloet ER, Lightman SL. Proteasome-dependent down-regulation of activated nuclear hippocampal glucocorticoid receptors determines dynamic responses to corticosterone. Endocrinology. 2007; 148(11): 5470-5477. doi: 10.1210/en.2007-0585

[104] Connell P, Ballinger CA, Jiang J, Wu Y, Thompson LJ, Hohfeld J, et al. The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. Nat Cell Biol. 2001; 3(1): 93-96. doi: 10.1038/35050618

[105] Wang X, DeFranco DB. Alternative effects of the ubiquitin-proteasome pathway on glucocorticoid receptor down-regulation and transactivation are mediated by CHIP, an E3 ligase. Mol Endocrinol. 2005; 19(6): 1474-1482. doi: 10.1210/me.2004-0383

[106] Qian SB, McDonough H, Boellmann F, Cyr DM, Patterson C. CHIP-mediated stress recovery by sequential ubiquitination of substrates and Hsp70. Nature. 2006; 440(7083): 551-555. doi: 10.1038/nature04600

[107] Morales JL, Perdew GH. Carboxyl terminus of hsc70-interacting protein (CHIP) can remodel mature aryl hydrocarbon receptor (AhR) complexes and mediate ubiquitination of both the AhR and the 90 kDa heat-shock protein (hsp90) *in vitro*. Biochemistry. 2007; 46(2): 610-621. doi: 10.1021/bi062165b

[108] Kundrat L, Regan L. Identification of residues on Hsp70 and Hsp90 ubiquitinated by the cochaperone CHIP. J Mol Biol. 2010; 395(3): 587-594. doi: 10.1016/j.jmb.2009.11.017

[109] Yan F, Gao X, Lonard DM, Nawaz Z. Specific ubiquitin-conjugating enzymes promote degradation of specific nuclear receptor coactivators. Mol Endocrinol. 2003; 17(7): 1315-1331. doi: 10.1210/me.2002-0209

[110] Hay RT. SUMO: A history of modification. Mol Cell. 2005; 18(1): 1-12. doi: 10.1016/j.molcel.2005.03.012

[111] Gareau JR, Lima CD. The SUMO pathway: Emerging mechanisms that shape specificity, conjugation and recognition. Nat Rev Mol Cell Biol. 2010; 11(12): 861-871. doi: 10.1038/nrm3011

[112] Poukka H, Karvonen U, Janne OA, Palvimo JJ. Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). Proc Natl Acad Sci U S A. 2000; 97(26): 14145-14150. doi: 10.1073/pnas.97.26.14145

[113] Le Drean Y, Mincheneau N, Le Goff P, Michel D. Potentiation of glucocorticoid receptor transcriptional activity by sumoylation. Endocrinology. 2002; 143(9): 3482-3489. doi: 10.1210/en.2002-220135

[114] Golebiowski F, Matic I, Tatham MH, Cole C, Yin Y, Nakamura A, et al. System-wide changes to SUMO modifications in response to heat shock. Sci Signal. 2009; 2(72): ra24. doi: 10.1126/scisignal.2000282

[115] Tian S, Poukka H, Palvimo JJ, Janne OA. Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor. Biochem J. 2002; 367(Pt 3): 907-911. doi: 10.1042/BJ20021085

[116] Holmstrom S, Van Antwerp ME, Iniguez-Lluhi JA. Direct and distinguishable inhibitory roles for SUMO isoforms in the control of transcriptional synergy. Proc Natl Acad Sci U S A. 2003; 100(26): 15758-15763. doi: 10.1073/pnas.2136933100

[117] Iniguez-Lluhi JA, Pearce D. A common motif within the negative regulatory regions of multiple factors inhibits their transcriptional synergy. Mol Cell Biol. 2000; 20(16): 6040-6050. doi: 10.1128/MCB.20.16.6040-6050.2000

[118] Holmstrom SR, Chupreta S, So AY, Iniguez-Lluhi JA. SUMO-mediated inhibition of glucocorticoid receptor synergistic activity depends on stable assembly at the promoter but not on DAXX. Mol Endocrinol. 2008; 22(9): 2061-2075. doi: 10.1210/me.2007-0581

[119] Carbia-Nagashima A, Gerez J, Perez-Castro C, Paez-Pereda M, Silberstein S, Stalla GK, et al. RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1alpha during hypoxia. Cell. 2007; 131(2): 309-323. doi: 10.1016/j.cell.2007.07.044

[120] Druker J, Liberman AC, Antunica-Noguerol M, Gerez J, Paez-Pereda M, Rein T, et al. RSUME enhances glucocorticoid receptor SUMOylation and transcriptional activity. Mol Cell Biol. 2013; 33(11): 2116-2127. doi: 10.1128/MCB.01470-12

[121] Kotaja N, Karvonen U, Janne OA, Palvimo JJ. The nuclear receptor interaction domain of GRIP1 is modulated by

covalent attachment of SUMO-1. J Biol Chem. 2002; 277(33): 30283-30288. doi: 10.1074/jbc.M204768200

[122] Paakinaho V, Kaikkonen S, Makkonen H, Benes V, Palvimo JJ. SUMOylation regulates the chromatin occupancy and anti-proliferative gene programs of glucocorticoid receptor. Nucleic Acids Res. 2013. doi: 10.1093/nar/gkt1033

[123] Gottlicher M, Heck S, Doucas V, Wade E, Kullmann M, Cato AC, et al. Interaction of the Ubc9 human homologue with c-Jun and with the glucocorticoid receptor. Steroids. 1996; 61(4): 257-262. doi: 10.1016/0039-128X(96)00032-3

[124] Kaul S, Blackford JA Jr, Cho S, Simons SS Jr. Ubc9 is a novel modulator of the induction properties of glucocorticoid receptors. J Biol Chem. 2002; 277(15): 12541-12549. doi: 10.1074/jbc.M112330200

[125] Cho S, Kagan BL, Blackford JA Jr, Szapary D, Simons SS Jr. Glucocorticoid receptor ligand binding domain is sufficient for the modulation of glucocorticoid induction properties by homologous receptors, coactivator transcription intermediary factor 2, and Ubc9. Mol Endocrinol. 2005; 19(2): 290-311. doi: 10.1210/me.2004-0134

[126] Kotaja N, Aittomaki S, Silvennoinen O, Palvimo JJ, Janne OA. ARIP3 (androgen receptor-interacting protein 3) and other PIAS (protein inhibitor of activated STAT) proteins differ in their ability to modulate steroid receptor-dependent transcriptional activation. Mol Endocrinol. 2000; 14(12): 1986-2000. doi: 10.1210/me.14.12.1986

[127] Tirard M, Jasbinsek J, Almeida OF, Michaelidis TM. The manifold actions of the protein inhibitor of activated STAT proteins on the transcriptional activity of mineralocorticoid and glucocorticoid receptors in neural cells. J Mol Endocrinol. 2004; 32(3): 825-841. doi: 10.1677/jme.0.0320825