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## **Diversity of glucocorticoid receptor signaling: molecular mechanisms and therapeutic implications**

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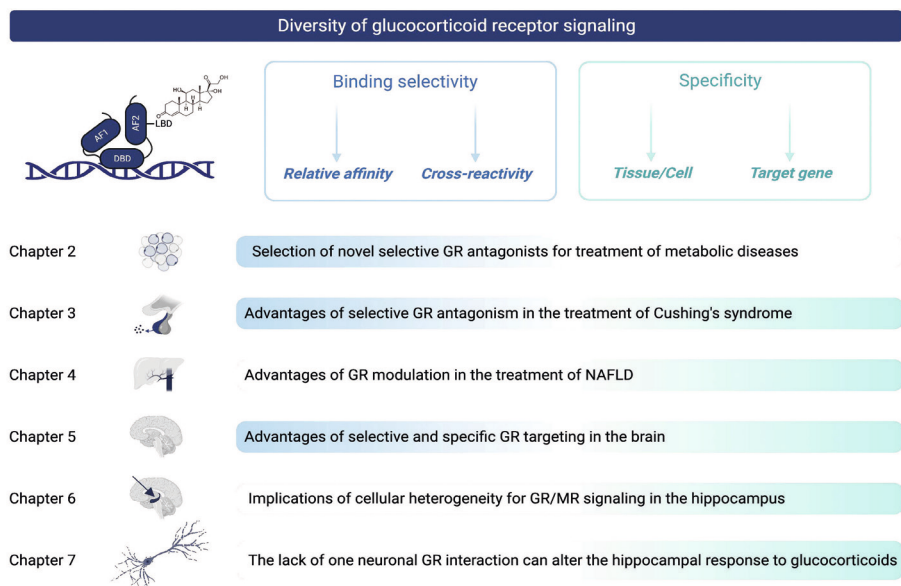
# 8

## General discussion and perspectives



## DISCUSSION AND PERSPECTIVES

From an evolutionary perspective, stress is an adaptive system that is necessary to generate appropriate responses to stochastic and unpredictable events, and cope accordingly with the environment. The physiological response to stress has been remarkably conserved in vertebrate evolution [1, 2]. However, the threats to our internal “equilibrium” have changed between our ancestral environments and our current modern societies, and the demands for survival have evolved [3, 4]. The glucocorticoid receptor (GR) is a timeless component of stress adaptation, as it is at the intersection between the environmental stressors (*i.e.*, physical, or psychosocial) and the genome. Therefore, the GR represents a valuable therapeutic target in stress- and glucocorticoid-related disorders. This thesis provides new insights into the molecular mechanisms underlying GR signaling in metabolic diseases and brain function and highlights the promise and importance of selectivity in novel GR targeting treatments. Optimal selective targeting of the GR requires 1) a relatively high receptor affinity, 2) the lack of cross-reactivity with other steroid hormone receptors, and preferably limits treatment adverse effects by inducing 3) tissue- and cell-specific outcomes which involve 4) only a subset of genes and pathways (**Fig. 1**).



**Figure 1. Overview of the thesis chapters.** Diversity of glucocorticoid receptor signaling, its molecular mechanisms and the therapeutic implications in metabolic and psychiatric diseases. **Abbreviations:** AF1, activation function 1; AF2, activation function 2; LBD, ligand-binding domain; DBD, DNA-binding domain; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; NAFLD, non-alcoholic fatty liver disease.

We developed a preclinical pipeline to identify novel selective GR antagonists with beneficial properties in metabolic diseases (**chapter 2**), and we demonstrated that selective GR antagonism by relacorilant only induces a modest disinhibition of the hypothalamic-pituitary-adrenal (HPA) axis activity compared to mifepristone, which represents a considerable advantage in the treatment of Cushing's syndrome (**chapter 3**). In **chapter 4** and **chapter 5**, we explored the molecular mechanisms underlying the beneficial effects of selective GR modulation in non-alcoholic fatty liver disease, and in stress-related psychiatric disorders that are often associated with disruption in hippocampal GR signaling. To better comprehend glucocorticoid effects in the hippocampus, we created an atlas in the mouse hippocampus that recapitulated the cell-specific expression of genes involved in glucocorticoid signaling (**chapter 6**). Finally, we investigated the effects of the neuronal absence of the GR interacting protein UBE3A on hippocampal GR signaling and the consequences in the context of UBE3A-deficient mice with Angelman syndrome (**chapter 7**) (**Fig.1**).

### **Main components of selective glucocorticoid receptor targeting**

#### *Binding affinity and pharmacodynamics*

The first prerequisite for a selective GR antagonist is high binding affinity, expressed as a low dissociation constant ( $K_d$ ), typically in the low nM range. In our current selection of selective GR antagonists, the first evaluated parameter is the half-maximal inhibitory concentration (IC<sub>50</sub>) as determined in competition with a GR agonist (cortisol or dexamethasone) *in vitro* using a tyrosine aminotransferase (TAT) reporter assay. This gives an indication of the potency of the compound to antagonize GR signaling [5]. Mifepristone is currently the most potent GR antagonist, with a  $K_d$  of 3.0 nM and an IC<sub>50</sub> of 0.4 nM. In comparison, the selective GR antagonist relacorilant described in the thesis has a slightly lower GR affinity ( $K_d$  of 7.2 nM) and inhibiting potency (IC<sub>50</sub> of 2 nM) [6, 7]. Relacorilant is so far the second most potent GR antagonist we characterized, it inhibited the expression of GR target genes in most metabolic tissues, which was associated with a relative high efficacy in preventing both corticosterone-induced hyperinsulinemia, immunosuppression (**chapter 3**) [8]. Despite being a good predictor of GR targeting efficacy, the  $K_d$  and TAT assay-based evaluation of selective GR antagonists lacks specificity. For instance, CORT108297 is a selective GR modulator with a very high affinity for the GR ( $K_d = 0.9$  nM) that shows efficacy in the TAT assay (IC<sub>50</sub> ~ 120 nM). It previously showed GR antagonistic properties in an Alzheimer's disease model by blocking the glucocorticoid-mediated increase in hippocampal amyloid- $\beta$ , but acted as a GR agonist in memory consolidation in healthy animals [9, 10]. Similarly, the GR modulator CORT118335 ( $K_d = 8$  nM) acted as a GR antagonist in memory

consolidation while displaying GR antagonistic and partial agonistic properties in the liver (cf. **chapter 4**, **chapter 5**) [11–13].

The  $K_d$  value for a single functional readout by definition does not allow the distinction between selective GR antagonists and selective GR modulators. However, in competition with cortisol or dexamethasone, an IC50 in the lower nanomolar range seems to be a characteristic of the current best candidates for selective GR antagonism. For selective GR modulators – CORT108297 and CORT118335 – we found IC50s above 120 nM in our TAT reporter assay, indicating partial GR agonism. Together with the chemical structure and properties, the *in vitro* pharmacological screening of selective GR compounds does not allow the distinction between selective GR antagonists and selective GR modulators, which is necessary to identify the future therapeutic applications of the candidate compounds.

#### *Cross-reactivity with other steroid nuclear receptors*

The principal advantage of selective GR targeting is the lack of binding to other steroid hormone nuclear receptors. All the novel compounds described in this thesis do not bind to the receptors for progesterone (PR) or androgens (AR) [14]. In contrast, the non-selective GR antagonist mifepristone is also used as a PR antagonist for the purpose of medical abortion. In the context of GR targeting in female patients with hypercortisolism, mifepristone is associated with several adverse effects due to PR cross-reactivity, including irregular vaginal bleeding, and endometrial thickening [15, 16]. Mifepristone treatment causes disinhibition of the HPA axis and subsequently excessive secretion endogenous cortisol. This results in overactivation of the mineralocorticoid receptor (MR), which is associated with hypokalemia, hypertension, and edema [16–18]. In contrast with mifepristone, the selective GR antagonist relacorilant does not bind to the PR and only modestly disinhibits the HPA axis [6, 19]. In **chapter 3**, we showed that relacorilant had properties similar to the ones of mifepristone in the treatment of metabolic symptoms triggered by excessive glucocorticoid exposure [8].

It is clinically relevant to consider crosstalk between stress and sex hormones when targeting the GR to avoid sex-specific adverse side effects, but also because the GR can be a therapeutic target in pathologies with a higher prevalence in men or women. Selective GR antagonists are not exempt of sex-specific effects, likely because of the intrinsic differences between males and females in glucocorticoid-driven functions. For example, the metabolic effects associated with excessive glucocorticoid exposure can be – and often are – sex-dependent [20–24]: many features of the metabolic syndrome that predispose to the development of

cardiovascular diseases are either more pronounced in men or more common in women, and the sex hormone profile is often suggested as an important contributor to these sex differences [25, 26]. We observed such differences in our studies. For instance, short-term treatment with the selective GR antagonist CORT125329 in mice under high-fat diet lowered plasma lipids in female but not male mice, while short- and long-term treatment in male mice predominantly improved glucose tolerance (cf. **chapter 2**) [7].

The susceptibility to stress-related neuropsychiatric and neurodegenerative pathologies also differ between men and women [27–29]. Many of these disorders have been associated to alterations in hippocampus structure and function [30–34]. We have shown in **chapter 6** that the cell type-specific expression of the GR and MR substantially correlated with AR and PR mRNA in the mouse hippocampus, which suggests a possible crosstalk between these receptors in this brain region. The evaluation of GR signaling in the hippocampus after manipulation of sex hormone secretion can reveal to what extent sex plays a role in the hippocampal stress response.

Due to the structural and functional similarities of the GR and MR, it remains challenging to selectively target the GR without influencing MR activity. The GR modulator CORT118335 also acts as a low-affinity MR antagonist. A CORT118335-analogue – CORT125385 – showed comparable effects in the mouse liver but no MR cross-reactivity (unpublished data). This suggests that the therapeutic effects of CORT118335 in non-alcoholic fatty liver disease (NAFLD) described in **chapter 4** can be attributed to GR modulation. Similarly, the inhibitory effects of CORT118335 on GR signaling and memory consolidation in the hippocampus mimic those of mifepristone, and may therefore be attributed to GR modulation, rather than MR antagonism [11]. GR antagonism by mifepristone clearly has beneficial effects in some stress-related disorders like post-traumatic stress disorder [35–37], but it did not yet fulfill its expected potential as an antidepressant or antipsychotic treatment. One potential explanation its lack of MR antagonism [11, 38]. The MR plays a critical role in brain sensitivity to stress and glucocorticoid hormones [12]. The MR is largely occupied under basal hormonal conditions and is involved in the onset of stress-induced activity of HPA axis. In contrast, the GR is activated with higher hormonal levels and predominantly involved in the final stages of the stress response, including the memory imprinting (cf. **chapter 5**) [39, 40]. The hippocampus is involved in the regulation of the HPA axis, and shows high expression of both the MR and GR [41–43]. Therefore, it was previously proposed that the hippocampal imbalance between MR and GR signaling could underlie HPA axis dysregulation

associated with the susceptibility to stress-related psychiatric disorders such as depression or PTSD [44–48]. The role of MR in psychopathology is complicated by the fact that aldosterone binds to MR which may have very different effects than cortisol-bound MRs. Hyperaldosteronism – for example in Conn’s Disease – is linked to increased risk of psychopathology, whereas genetic variants of the MR activity have been reported to be protective in the pathogenesis of numerous stress-related psychiatric disorders [49–52]. It will be of interest to study the consequences of the combined GR and MR antagonism by CORT118335 in the treatment of stress-related psychiatric disorders in preclinical models.

In addition, the ongoing clinical evaluation of CORT118335 treatment to attenuate antipsychotic-induced weight gain may provide more insight in the effects of CORT118335 in the brain [53]. In conclusion, the selectivity of novel GR antagonists such as relacorilant represents a considerable advantage over mifepristone to prevent cross-reactivity with sex hormone receptors, and to subsequently limit sex-specific adverse side effects in metabolic diseases and psychiatric disorders. However, in the context of GR modulation in relation to psychopathology, the functional consequences of antagonism of both the GR and MR are more difficult to predict.

#### *Tissue specificity and pharmacokinetics*

The fact that selective GR targeting antagonists or modulators often have tissue-specific effects can be an important feature when aiming to reduce putative adverse effects of the treatment. For example, the selective GR antagonist CORT125281 showed strong GR antagonism in the liver, partial and gene-specific GR antagonism in the muscle and brown adipose tissue (BAT), and no effect in white adipose tissue (WAT) or the brain [54]. The lack of effects in the brain were explained by the poor brain penetrance of the compound, but the differences between metabolic tissues could not fully be explained by differences in CORT125281 pharmacokinetics. Similar to CORT125281, CORT125329 showed no GR antagonism in the WAT but had significant effects on BAT (cf. **chapter 2**). Upon CORT125329 treatment under high-fat diet feeding conditions, we observed a decrease in BAT tissue weight and an upregulation of UCP1 expression, which together indicate enhanced thermogenic activity. It is still unclear whether the regulation of thermogenic activity was caused by direct effects of CORT125329 on BAT, or whether other metabolic tissues (*e.g.*, liver or muscle) responded to the treatment and indirectly influenced BAT activity [7].



Regardless, the tissue specificity of selective GR antagonists is of clinical interest in the sense that it can limit side effects caused by undesirable molecular alterations in tissues not directly involved in the disease. The lack of effects in a certain tissue may also represent a therapeutic advantage at the systemic level. For instance, the lack of central GR antagonism by relacorilant could explain the modest disinhibition of the HPA axis observed in mice, as compared to mifepristone (cf. **chapter 3**) [8]. Mifepristone treatment strongly inhibits both central and peripheral negative feedback on the HPA axis negative feedback, and this results in excessive levels of circulating adrenocorticotrophic hormone (ACTH) and subsequently glucocorticoids [55]. In patients with endogenous Cushing's syndrome, long-term treatment with mifepristone resulted in a continuous elevation of circulating ACTH [56], while treatment with relacorilant was not associated with a significant increase in serum ACTH and cortisol concentrations [19]. Besides having side effects via MR activation, the elevated levels of cortisol upon mifepristone treatment work against the antagonist at the tissue level. In comparison, the relatively lower GR affinity of relacorilant may be compensated by its modest disinhibition of the HPA axis.

Profiling of drug targets has been demonstrated to be a good predictor of drug safety and efficacy, but is limited by the lack of resources to identify the relevant target tissues [57]. Tissue specificity is a dominant predictor of unforeseen adverse treatment effects [58], and the understanding of the biological mechanisms underlying genetic predisposition to diseases involves both tissue-shared and tissue-specific features [59]. Considering the pleiotropic functions of the GR, some GR-related diseases might benefit from GR targeting in several tissues, while other pathologies could be better treated with tissue-specific GR targeting. In the future of compound screening, it could be of interest to separate the potential applications based on compound degree of tissue specificity. For this purpose, it is important to further develop our current understanding of tissue-specific GR signaling and the GR regulatory network in health and disease.

## **Definition of glucocorticoid receptor regulatory network**

### *Cell specificity in health and disease*

Beyond their considerable therapeutic value, the selective GR compounds represent a valuable tool to decipher the molecular mechanisms underlying GR signaling. To successfully target the GR, it is important to consider the complexity of GR signaling which involves GR transcriptional protein partners and its target genes. The expression of GR and coregulatory proteins differ between tissues but also between cell types, which leads to cell-specific effects of GR ligands (**chapter 5**) [12]. The possibility to perform transcriptomic studies at the single cell level greatly

enhances our options to interpret the effects of glucocorticoid receptor ligands (**chapter 6**) [43].

As an example, CORT118335 treatment was previously shown to reduce liver lipid content in a mouse model for NAFLD, presumably by limiting lipid uptake and stimulating lipid efflux. This was associated with a consistent reduction in *Cd36* expression and an increase in *Mttp* expression [13]. *Cd36* is known to drive the onset of liver steatosis by stimulating fatty acid uptake and can contribute to NAFLD progression [60, 61]. In **chapter 4**, we analyzed public mouse and human liver scRNA-seq datasets to describe the heterogenous expression of genes involved in GR signaling and lipid metabolism in different liver cell types [62, 63]. The results showed that *Cd36* was predominantly expressed in endothelial cells. In contrast, *Mttp* was mostly expressed in mouse and human hepatocytes. We therefore hypothesized that the ability of CORT118335 to combine GR agonism and antagonism in the liver involved cell type-specific signaling mechanisms. In the brain, the use of scRNA-seq profiling allows large-scale comprehensive molecular classification of cell types and subregions, which can reveal qualitative and quantitative differences in nuclear receptor expression (cf. **chapter 5**) [12].

In **chapter 6**, we used scRNA-seq data from mouse hippocampus generated by the Allen Brain Institute to assess the cell type-specific expression of genes that potentially interact with GR and MR signaling [64]. Despite the lack of scRNA-seq data after glucocorticoid treatment, our results allowed the reinterpretation of glucocorticoid responsiveness in the adult mouse hippocampus [43]. The scRNA-seq technology offers unprecedented insight into the transcriptional cellular heterogeneity. In order to meet the level of complexity brought by recent developments in single-cell transcriptomics, numerous computational tools have been developed to assess gene regulatory networks [65–71]. However, this remains a dominant challenge in scRNA-seq analysis. For example, in the mouse hippocampus, we failed to identify GR and MR gene regulatory networks (GRNs). The limitation with steroid hormone receptors such as GR and MR is that they often act via enhancer elements in the DNA that are distant from their target gene promoters. For hippocampal target genes, an *in silico* interspecies screening of glucocorticoid-responsive genes showed that GR binding sites were commonly between 30kb downstream and 175kb upstream of the target gene transcription start site [43, 72]. The pipeline used in **chapter 6** to detect receptor binding sites was limited to 10kb down- and up-stream of gene transcription start sites [67], and therefore the GR and MR did not meet the selective criteria for GRN inference as less than 80% of their putative target genes showed receptor binding in their promoter region.

Nevertheless, the activity of other transcription factor GRNs provided the cellular context in which MR and GR can bind to chromatin, as exemplified by the cell-specific expression of the GRN of *Neurod2* that is relevant to identify MR target genes.

Our research on the cellular heterogeneity of GR signaling currently lacks the inclusion of disease and glucocorticoid treatment context to better comprehend the cell types and genes involved in GR signaling in physiology and pathology. Future research using single-cell multi-omics could reveal disease-specific alterations in GR signaling and further characterize the molecular mechanisms underlying successful targeting of the GR.

#### *The downstream regulatory network*

Beyond cell specificity, GR targeting also involves gene specificity and per definition selective GR modulators (and sometimes antagonists) differentially affect subsets of target genes. Examples from this thesis include the differential antagonistic potencies of relacorilant on *e.g.*, *Pomc* and *Fkbp5* in **chapter 3**, and the gene-specific regulation by CORT118335 in **chapter 4**. In **chapter 6**, we attempted to generate a list of genes that were consistently altered by glucocorticoid treatment in the hippocampus. We combined a published meta-analysis on glucocorticoid responsive genes in rat, mouse, and human brain tissue [73] with a RNA-seq dataset that we obtained in mouse hippocampus, and data from chromatin immunoprecipitation followed by sequencing (ChIP-seq) against GR and MR in rat hippocampus [74, 75].

This resulted in a list of 4609 genes either responsive to glucocorticoid treatment or associated with a receptor binding site. Only 2% of these genes were actually regulated by corticosterone in our mouse hippocampus RNA-seq dataset, including 19 genes that were consistent with the previously findings [73] and were associated with GR and/or MR binding in their promoter region [43]. A question that arises is what really defines a GR target gene?

The GR can bind in regions very distant from the target locus [72], it is therefore challenging to fully apprehend the direct GR regulatory network. The nucleosome positioning and occupancy is organized by the combinatorial action of transcription factors like the GR and other epigenetic factors that regulate DNA accessibility [76, 77]. In this regard, open chromatin profiling has been widely used to identify regulatory elements. Active regulatory elements such as transcription start sites and enhancers that are accessible for transcription factor binding can be marked by histone-3-lysine-27 acetylation (H3K27ac), thereby allowing the assessment of the activity state of both proximal and distal target genes [77–80]. In **chapter 4**,

we assessed the global GR colocalization with H3K27ac upon cortisol, CORT118335, and mifepristone treatment using immunofluorescence in HepG2 cells. This allowed us to observe quantitative differences of GR binding to active DNA regions between different treatment regimens. Unfortunately, this global approach does not allow locus-specific investigations. To add a qualitative component to this type of analysis, it is possible to assess the intersection between GR binding and the accessible DNA loci by combining ChIP-seq against GR and H3K27ac, and/or an assay for transposase-accessible chromatin using sequencing (ATAC-seq) [81]. These combined approaches in an U2OS cell line showed that GR binding sites were more accessible after dexamethasone treatment, as compared to basal conditions, and that enhancer activity for GR-occupied regions increased upon dexamethasone treatment [82]. This is in line with our observations in HepG2 cells, wherein cortisol treatment significantly increased both H3K27ac and GR colocalization with H3K27ac, suggesting an increase in GR binding to open active chromatin (cf. **chapter 4**). Another way to appreciate the extent of GR activation of transcriptionally active regions (enhancers) is to perform Hi-C analyses. The Hi-C technology allows the analysis of genome architecture by identifying genome-wide long-range interactions between DNA loci. For instance, a recent study in rats revealed addiction-associated transcription repression at regulatory enhancers known to be recognized by the GR [83].

The transcriptional signature of glucocorticoids and GR ligands is highly context dependent. In the context of diet induced NAFLD in mice, CORT118335 consistently reduced the expression of *Cd36* in the liver. In contrast *Cd36* was only modestly altered by CORT118335 treatment in mice under low fat diet (**chapter 4**). In **chapter 7**, our RNA-seq analysis provided genome-wide evidence of an altered hippocampal transcriptome in mice with Angelman syndrome compared to wild-types controls following acute corticosterone exposure.

The results showed that the disease context can significantly alter GR signaling and change the subset of genes that is subsequently affected by excessive glucocorticoid exposure. In the same study, no significant transcriptomic differences were found in the hippocampus of Angelman syndrome mice after continuous corticosterone treatment, as compared to the control mice. This suggests a differential GR response to acute and continuous corticosterone treatment in the brain, as expected based on previous findings with acute and chronic stress paradigms [84, 85]. Therefore, the context-dependence of GR signaling is also defined by the dosage and duration of the treatment.

*The interactome at the basis of the genomic diversity of GR signaling*

The transcriptional activity of the GR depends on numerous interactions with transcription factors, coregulators and chromatin remodelers, as described in **chapter 1**. It was previously shown that the extent of agonism and antagonism on GR-mediated gene expression can depend on transcriptional cofactor availability and ratios, which can differ in certain tissues or cell types [12, 14, 43, 54, 86, 87]. In **chapter 3**, we showed that despite crossing the blood brain barrier, relacorilant did not antagonize GR target genes in the hippocampus while mifepristone did [8]. Relacorilant diverged from mifepristone in terms of GR transcriptional coregulator recruitment *in vitro*. Our results suggested that the antagonistic properties of mifepristone on the GR may rely on interaction with nuclear receptor corepressor 1 and 2, while GR antagonism by relacorilant seems to be associated with a lack of coactivator recruitment. Although we had no direct evidence of the causal effects of the GR interactome on the differential effects of relacorilant versus mifepristone, we can speculate that the lack of GR corepressor recruitment with relacorilant limits its GR antagonistic potential in the brain while the lack of coactivators is sufficient for effective peripheral GR antagonism.

In the context of CORT118335 treatment, we found that the GR interactome in human HepG2 cells lacked many coactivator proteins but showed an enrichment in proteins involved in cytoskeleton organization (cf. **chapter 4**). This subset of proteins includes tubulins, which are involved in GR mobility and its transport from the cytosol to the cell nucleus [88–91] and thereby define GR subcellular distribution [92, 93]. Even though our immunohistochemistry showed nuclear translocation of GR after CORT118335, this suggests that CORT118335 treatment could interfere with GR cell trafficking and induce a unique GR distribution in the cell nucleus. Previous studies suggested that GR ligand affinity was a major determinant of GR mobility, and that this process was cell-type specific. The assessment of GR mobility over time could be used to confirm whether GR nuclear translocation is impaired after CORT118335 exposure.

The CORT118335-induced GR interactome lacked proteins involved in gene transactivation and chromatin remodeling, like SMARCD1 (BAF60A). SMARCD1 mediates critical interactions between nuclear receptors and the BRG1-dependent SWI/SNF chromatin remodeling complex, and GR-driven chromatin remodeling is known to require the BRG1 SWI/SNF complex [94, 95]. GR coregulators and chromatin remodelers are shared with other nuclear receptors, and this can imply competition for coregulator protein recruitment. SMARCD1, may be limiting for some transcriptional processes. In one previous study, SMARCD1 overexpression

improved hepatic steatosis in male mice, and this was shown to be mediated by PGC1 $\alpha$  induction of SMARCD1 recruitment to PPAR $\alpha$  target loci involved in the maintenance liver lipid homeostasis [96]. PPAR $\alpha$  in turn, was previously shown to inhibit GR-driven gene transactivation by interfering with GR protein recruitment [97]. A possible scenario is that upon CORT118335 treatment, SMARCD1 recruitment by PPAR $\alpha$  is facilitated by the lack of competition by the GR, indirectly promoting lipid catabolism.

In the mouse hippocampus, GR was highly expressed in oligodendrocytes, astrocytes, microglia, and endothelial cells (**chapter 6**). GR function in glial cell types has previously been established by using mouse models with cell type-specific knockout [98–100]. Glucocorticoids previously displayed direct and indirect effects in these non-neuronal cell types in rodents and humans [101–104]. Specifically, microglial cells are very responsive to stress and glucocorticoids, and have been reported to play an important role in synaptic plasticity [105, 106]. Interestingly, the GR signaling repertoire in microglia is unique for the brain, since steroid receptor coactivator-1 is barely expressed, and steroid receptor coactivator-2 (SRC-2) is highly expressed. This is analogous to peripheral immune cells where GR effects predominantly rely on SRC-2 presence in the GR transcriptional complex [107, 108]. A cell type-specific coregulator repertoire may allow more selective targeting of GR using selective receptor modulators that distinguish between downstream signaling pathways [109, 110]. For instance, treatment with the selective GR modulator CORT108297 in an epilepsy model was shown to limit reactive microgliosis in the mouse dentate gyrus without affecting an increase in astrogliosis [111]. Finally, in **chapter 7**, the neuronal absence of the GR coregulator UBE3A in mice with Angelman syndrome led to the alteration of GR signaling in the hippocampus. Altogether, these observations suggest that GR interactome can define GR signaling and underlie the tissue, cell, gene, and context specificity. Future research on GR interactome could further define the transcriptional complex compositions which underlie stress adaptation or maladaptation.

### **Concluding remarks**

According to Hans Selye, “adaptation in itself is not pathogenic, but an indispensable physiological defense reaction to damage as such [...] It is not stress that kills us, it is our reaction to it”. Selective GR targeting represents a therapeutic strategy to dampen the damaging consequences of a maladaptive stress response or glucocorticoid excess. In this thesis, we discussed the importance of GR selectivity, the potentially beneficial effects of selective GR antagonists and selective GR modulators, and the complexity of the genomic and epigenomic events underlying

GR transcriptional regulation. The future of selective GR targeting holds many promises in the treatment of glucocorticoid- and stress-related metabolic and psychiatric disorders. This will require more experimentation to further improve our understanding of the diversity of GR signaling.

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