Chapter 15
Animal Models of Altered Glucocorticoid Signaling

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Abstract In this chapter we will review genetically engineered mice with alterations in glucocorticoid signaling. Most of the mice involve direct alterations to the glucocorticoid receptor locus, but we will touch briefly on other relevant models including 11-β-HSD transgenics which alter tissue levels of ligand as well as mice with glucocorticoid excess. Of course, the number of mice with mutations in genes such as GR targets and transcriptional coregulators is beyond the scope of this chapter.

Keywords Altered glucocorticoid signaling • Animal models • 11-β-HSD transgenics • Hypomorphs • GR-null mice • Tissue specific KO • Tissue-specific transgenics

Version 1.0: Hypomorphs

The scientific community was quite eager to know the function of the glucocorticoid receptor in vivo. Transgenic mice were introduced in 1981 [1], but gene targeting via homologous recombination was not introduced until 1990 [2] and did not become commonplace until the mid-1990s. GR was cloned in 1985 [3–5]. Therefore, in order to probe the function of GR in vivo in the early 1990s, multiple groups created transgenic animals with transgenes encoding antisense for GR coding sequence, thereby creating in essence a "tissue specific knockdown" governed by the promoter driving the anti-sense construct. The first paper expressed a rat antisense GR sequence under the control of a human neurofilament gene promoter. These mice were obese, had decreased but detectable glucocorticoid binding and GR mRNA in the brain, mildly increased serum corticosterone and very increased ACTH levels [6]. Another group used a similar approach to knockdown GR with a transgene encoding 3′ UTR rat GR antisense driven by lck, an early T-cell specific promoter. These mice had small

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thymi and had decreased number of thymocytes due to decreased differentiation of mature thymocytes as well as increased apoptosis [7].

Version 2.0: Whole Body Knockouts

GR-null mice have been generated separately from several different groups [8–10]. GR-null mice are born at the expected Mendelian frequency, but die within hours of birth due to respiratory difficulties and defects in lung maturation. This finding is concordant with clinical observations showing infants born prematurely suffer from similar defects in lung maturity if mothers are not given glucocorticoids shortly before birth. In addition, in GR-null mice, circulating ACTH and corticosterone levels are high due to loss of negative feedback. The elevated corticosterone does not activate GR in these animals (there is none), but would be predicted to activate MR and cause a syndrome of apparent mineralocorticoid excess. This phenomenon has been described in persons with resistance to glucocorticoid hormone [11]. GR-null mice also have cortical adrenal hyperplasia, presumably from increased ACTH levels stimulating intact ACTH receptors (MC2R) in the adrenal cortex. The role of GR in the adrenal chromaffin lineage has been subject to reinterpretation over the years. GR-null mice were originally reported to be missing adrenal medullae [8], but more careful studies showed the existence of adrenal chromaffin cells in normal numbers with the more specific phenotype of conversion of adrenergic to noradrenergic cells due to loss of GR-mediated activation of the enzyme PNMT (Phenylethanolamine *N*-methyltransferase), that converts noradrenaline to adrenaline). More recently, a study of tissue-specific loss of GR in noradrenergic cells (using the cre-lox system and a dopamine beta hydroxylase driven cre line) revealed loss of chromaffin cells as adults [12]. The synthesis of these findings is that GR is not required for the formation of adrenal chromaffin cells but does help maintain these cells into adulthood. An attempt to rescue neonatal mortality due to the lung phenotype by crossing GR-null mice to transgenic mice expressing GR under the SP-C promoter was unsuccessful [13]. GR-null mice have a defect in epidermal differentiation and skin barrier function [14]. Because neonatal mortality has precluded analysis of adult GR-null animals, several investigators have alternatively studied GR-null cells in vitro, in transplant experiments, GR heterozygous mice in vivo [10] and later tissue specific null mice. GR-null erythroid progenitors have decreased erythropoiesis in vitro [15]. In an elegant set of experiments it was demonstrated that E18.5 GR-null mice have a disorganized endocrine pancreas. GR-null pancreas appeared normal at E15.5 and the disorganization that occurred between E15 and E18.5 could be rescued by transplanting the pancreas under the kidney capsule of a GR expressing mouse. The conclusion of these experiments was the disorganization of the GR-null pancreas was an indirect effect [16].

Version 3.0: Tissue Specific KO and Tissue-Specific Transgenics

The advent of cre lox technology allows specific deletion of gene products from tissues or cell types of interest. A thorough review of the subject can be found here [17]. Briefly, a critical region of the gene of interest is modified to be flanked by 34 base pair loxP sites, which are introduced by homologous recombination in such a manner so as not to disrupt gene function. So called "floxed" (flanking loxP mice) are then crossed to transgenic mice expressing the bacteriophage cre recombinase driven by the promoter of interest. The cre recombinase is expressed only in the tissue of interest where it excises the DNA between the two loxP sites, resulting in a null allele.

The pleiotropic actions of glucocorticoids have made it difficult to draw direct links between specific gene activations with physiological observations. For example, glucocorticoids are known to cause osteoporosis, but this could be due to direct actions on bone, both osteoblasts and osteoclasts, but also indirectly through reductions in sex hormones, reductions in Growth Hormone, along with actions on the intestinal absorption of calcium. Another example is the known sarcopenic effects of glucocorticoids which could be explained by direct actions on muscle, but indirectly through suppression of the GH-IGF1 axis as well. Tissue specific deletion of GR has allowed a dissection of these pleiotropic actions to determine the contribution of cell-autonomous effects on respective phenotypes. Tissue-specific deletion of GR has confirmed effects on bone (osteoblast) and muscle are largely cell autonomous (see below). This is of clinical importance as novel therapeutics with tissue-specific delivery of GR modulators becomes a possible avenue in the future [18–20]. Glucocorticoid Receptor has been deleted in many different tissues. We do not have sufficient room to include all of these mice, but rather describe a handful and direct the reader to MGI for a complete listing. With respect to GR several different floxed mice have been generated with loxP sites flanking different exons of GR genomic locus [21–25]. Recently a comparison between exon 2 floxed mice and exon 3 floxed mice was used using the Sim1-cre mice with expression in the hypothalamus. Exon 3 mice gave an interesting expected phenotype of Cushing's syndrome (due to loss of GR-mediated negative feedback on CRH in the hypothalamus, but exon 2 flanked mice crossed to the same cre transgenic mouse did not display the phenotype. This may be due to inefficient excision of exon 2 in exon 2 floxed mice bearing certain cre transgenes. Alternatively, there may be truncated GR after exon 2 excision that can retain some transcriptional activity [26]. These observations do not derail previous results obtained with exon 2 floxed mice, as some cre lines do result in complete excision and loss of function when exon 2 is excised. However, given the potential for residual function with exon 2, we anticipate greater use of exon 3 floxed mice for future studies.

Lung

Since GR-null mice died due to defects in lung maturation, it was of great interest to know which cell type was responsible for the phenotype. Unfortunately there has not been consensus among investigators in this area. One group found that mesenchymal expression of GR governed the phenotype since mesenchymal-specific deletion of GR using col1-cre or dermo1-cre mice recapitulated the neonatal mortality phenotype [27, 28] but deletion using epithelial SPC-cre did not have a phenotype whereas another group observed decreased viability with an epithelial specific GR KO mouse that was generated by crossing GR floxed mice to mice carrying SPC-rtTA and tetO-Cre transgenes [29]. So, there is disagreement as to the necessity of GR in these cell types. As mentioned above, attempts to rescue the GR-null mouse by crossing to a transgenic expressing GR under the control of surfactant protein C regulatory elements were unsuccessful. It would be interesting to attempt a similar rescue with a mesenchymal targeted GR transgene. However, it is not clear whether a GR-null mouse rescued from the lung phenotype would survive given defects in other cell types or gain of function phenotype from excessive action on the mineralocorticoid receptor (syndrome of apparent mineralocorticoid excess).

Skin

The skin phenotype of global GR-null mice was described above. This phenotype is recapitulated in keratinocyte specific GR KO mice generated using cre lox technology and keratin14-cre mice [30]. These epidermal GR KO mice also have increased susceptibility to chemical induced skin tumors [31]. Overexpression of GR in transgenic mice driven by the keratin5 promoter results in abnormal skin including hypoplasia and impaired hyperplastic response to topical TPA [32].

Cardiovascular System

GR action in the distal nephron is not critical for glucocorticoid mediated hypertension [33] as mice with GR deleted in the distal nephron (Ksp-cre) had a hypertensive response to dexamethasone similar to WT mice. In contrast, mice with endothelial specific GR KO mice were created by using tie2-cre mice. Endothelial GR KO mice are resistant to dexamethasone mediated hypertension and have increased sensitivity to hemodynamic shock associated with sepsis as mimicked by systemic LPS injections [34]. This effect is thought to be mediated by loss of negative regulation of iNOS in maintaining vascular tone. A conditional induction of a GR transgene in the heart resulted in arrhythmia [35]. Deletion of GR in the heart (α-MHC-cre) results in

death at age 6 months from heart failure, indicating a previously unknown role for GR signaling in the heart [22]. These findings have potential clinical implications for the development of GR antagonists in metabolic disease. In addition global GR-null embryos were found to have decreased cardiac function [36].

Muscle

Muscle specific deletion of GR was accomplished using muscle creatine kinase-cre mice. Muscle specific GR KO mice were completely protected from dexamethasone mediated muscle atrophy [37] indicating this is a cell autonomous process. Induction of the atrogins MURF1 and MAFbx1 was abrogated in muscle specific GR KO mice treated with glucocorticoid. This was in contrast to the lack of effect seen in muscle-specific GRKO mice on muscle atrophy and atrogin gene activation seen with sciatic nerve transection. There was a partial effect on both atrogin gene induction and muscle atrophy seen with nutritional deprivation suggesting that glucocorticoids combine with other signals in that paradigm. A separate group has shown muscle GR is required for muscle atrophy following either chemotherapy or endotoxin [38, 39]. These reports did not indicate the contribution of muscle GR to whole body changes in glucose homeostasis, but may be the subject of future studies.

Gastrointestinal System

GR is dispensable for the development of the intestine as GR-null intestines are morphologically normal [40]. However, the perinatal death of GR-null mice precluded examination of GR in adult intestine. Enterocyte specific GR KO mice were created using villin-cre. Intestine-specific GR KO mice had loss of dexamethasone-stimulated intestinal glucose absorption that was thought to be mediated by transcriptional activation of genes encoding glucose transporters [41]. Recently, it was shown dexamethasone caused gastroparesis in wild type mice. This effect was not mediated by enterocytes as it was preserved in enterocyte specific GR KO animals (villin-cre). Rather, the authors believed it is due to loss of NO signaling in the stomach [42].

Immune System

Because GR-null animals die at birth one must use either tissue-specific knockouts or alternatively one can isolate fetal liver from GR-null animals and perform bone marrow transplants or perform bone marrow transplants from a tissue-specific KO to narrow down the cell type. Such bone marrow transplants were performed to show that macrophage GR was dispensable for atherogenesis in LDLR-null mice, but

macrophage GR contributed to vascular calcification [43]. Glucocorticoids are effective in multiple sclerosis and appear to act on T-cells in mouse models of autoimmune encephalitis [44] as evidenced by T-cell specific (lck) and macrophage specific KO (LysM-cre) mice. In a contact hypersensitivity model myeloid cells seemed to be the predominant mediator of glucocorticoid effects as myeloid specific GR KO mice (LysM-cre) were resistant to the anti-inflammatory effects of dexamethasone whereas T-cell (lck-Cre) and keratinocyte (K14cre-ER following tamoxifen injection) were unaffected [45]. T-cells were identified as the critical site of action for the therapeutic effects of glucocorticoid in an antigen-induced model of rheumatoid arthritis [46].

Bone

One group found osteoblast specific deletion of GR (Runx2-cre) rendered mice resistant to glucocorticoid-induced osteoporosis whereas deletion of GR in osteoclasts (LysM-cre) had no effect [47]. This same group did not see any defects in bone development as assessed by GR-null mice analyzed at E18.5 and also reported complete sensitivity of GR dim mice (see below) to glucocorticoid induced osteoporosis, implicating GR monomers in this process. Another group has implicated an anabolic function of glucocorticoids in bone development from the phenotype of transgenic mice expressing the glucocorticoid inactivating 11-β-HSD2 in osteoblasts [48]. Another group showed that action of GR in osteoclasts was the dominant determinant on the bone phenotype using a similar LysM-cre breeding strategy to create osteoclast deficiency of GR [49]. From these studies it is clear GR action on the osteoblast is critical for glucocorticoid induced osteoporosis, but there may be a role in the osteoclast as well. The actions of GR mediating osteoporosis do not require functional dimers. This has implications for the possible development of selective GR modulators as treatments for osteoporosis or to be used in conjunction with another glucocorticoid to attenuate glucocorticoid induced osteoporosis.

Tissues Involved in Metabolism

Deletion of GR in hepatocytes using albumin-cre resulted in neonatal mortality with incomplete penetrance [50]. These mice displayed fasting hypoglycemia and amelioration of streptozotocin induced diabetes [51]. The growth impairment in these mice phenocopied liver-specific deletion of Stat5 using the same cre line. The authors went on to show via microarrays that GR and Stat5 regulate similar sets of genes in the liver [52]. Deletion of GR in the liver with a different albumin-cre displayed no such viability defects. In addition, the latter mice had normal response to hepatectomy mediated hepatic regeneration [53]. The discrepant data with respect to viability is likely due to the timing of the two liver-specific cre activations, with early deletion being responsible for mortality and runting. The albumin-cre lines used in these studies are similar, but made by different investigators. The early

expressing albumin-cre that resulted in mortality and a growth phenotype contained additional alpha fetoprotein enhancer elements which may explain its earlier expression. Alternatively the timing of expression could have been affected by the genomic integration site. Liver-specific GR KO mice have also demonstrated a role for GR in bile acid homeostasis [54]. Specific deletion of GR in pancreatic beta cells using RIP-cre had no effect on alpha or beta cell mass whereas deletion of GR in the precursor, using Pdx1-cre resulted in a doubling of beta cell mass [55]. Furthermore, as noted above GR-null embryos have disorganized pancreata.

CNS and HPA Axis

Glucocorticoids are known to cause negative feedback on hypothalamic CRH and pituitary ACTH secretion and the phenotypes of tissue-specific GR-null mice are informative as to the relative contribution of these sites. Mice with neuron specific deletion of GR (nestin-Cre) displayed Cushing's syndrome due to loss of negative feedback at the hypothalamic CRH producing neurons. These mice also had reduced anxiety [25]. The phenotype of this mouse is similar to the CRH-Tg mouse (see below) as well as the mice with deletion of GR in the hypothalamus using Sim1-cre and to the phenotype of mice with the (-120) mutation in the CRH promoter (see below), all resulting in loss of negative feedback at the level of the hypothalamus. Mice with loss of GR in the ACTH producing cells of the pituitary (POMC-cre) have elevated corticosterone levels in the first week of life, but normal peak and nadir corticosterone levels as adults [56]. Pituitary GR KO mice did have impaired suppression of corticosterone in a dexamethasone suppression test. In contrast deletion of GR in both the pituitary and hypothalamus (calcium/calmodulin-dependent protein kinase II alpha-cre) results in death in the first week of life from an advanced form of Cushing's syndrome [57]. Therefore, loss of pituitary negative feedback has modest effects, loss of the hypothalamic site results in Cushing's syndrome but is compatible with life, but complete loss of negative feedback is not. Given the role of stress on psychiatric conditions a number of GR cell type specific KO mice have been analyzed in behavioral paradigms. GR in dopamine responding neurons (dopamine receptor 1-cre) was found to be a critical mediator of social aggression whereas GR in dopaminergic neurons (dopamine active transporter, DAT-cre) was dispensable. Mice subject to social aggression show characteristic behaviors which were abolished in mice lacking GR in dopaminoceptive neurons [58]. Mice with deletion of GR in dopaminoceptive neurons (D1R-cre) self-administered less cocaine than wildtype mice, but no effect was seen when GR was deleted from dopaminergic neurons (DAT-cre) [59]. GR has been found to be a mediator of MPTP-induced Parkinsonism in microglia. Microglial GR KO mice (LysM-cre) had more death of dopaminergic neurons following MPTP-insult, whereas the loss was not effected by deletion of GR from dopaminergic neurons (DAT-cre) [60]. Microglia from microglia GR KO mice also had increased activation, consistent with a role for glucocorticoids in regulating microglial activation status as an anti-inflammatory.

Other Tissues

Deletion of GR in the mammary epithelium (whey acidic protein-cre) had no effect on lactation, but there was a modest defect in glandular proliferation during pregnancy [61]. Deletion of GR in sertoli cells (using anti-Mullerian hormone-cre) resulted in decreased sertoli cell number, circulating FSH and secondarily decreased LH and abnormal Leydig cell morphology, but these mice were fertile [62]. As mentioned above, deletion of GR in noradrenergic cells (using dopamine beta hydroxylase cre) resulted in decreased numbers of adrenal chromaffin cells in adults.

Version 4.0: Qualitative Differences (Point Mutants)

The most well studied GR knockin mouse is the dim mouse (MGI:1931329). Site directed mutagenesis studies identified this mutation in vitro as inhibiting transactivation while leaving repression intact [63]. Alanine to threonine mutation was generated by homologous recombination. Unlike GR-null animals, GR dim mice are viable [64]. However, we have observed most GRdim/dim mice on a C57/BL6 background die at birth [65]. Interestingly, viability is normal on a mixed (BALBC and C57) background (C.H., unpublished observation). This could be due to genetic modifiers affecting GR signaling. In addition, although the dim allele was originally described as being a loss of function allele for all transactivation targets [64], more detailed analysis in the age of genomics has uncovered that some targets are still activated by the dim allele and in addition the dim allele has some gain of function properties [66, 67]. In other words, a subset of genes is activated in a ligand dependent manner by the dim allele that is not normally activated by wildtype GR. These so called "rogue" genes have been identified in vitro [66, 68] as well as in vivo [69 and our unpublished results]. One potential explanation for gain of function properties of the dim allele is gene activation by an increased concentration of GR monomers in the absence of dimer formation. Therefore, though the dim mouse is a powerful tool to dissect whether biological effects are mediated by dimers experiments using them must be interpreted with this in mind.

GR dim mice had blunted response to anemia by phenylhydrazine and also did not increase red blood cell counts in response to hypoxia [15]. As opposed to wildtype mice, GR dim mice did not potentiate hippocampal calcium currents or enhance serotonin response in the hippocampus when treated with glucocorticoids [70]. Water maze experiments demonstrated that GR dim mice have impairments in spatial memory [71]. GR dimerization is required for inhibition of contact hypersensitivity by glucocorticoids [45]. The defects in skin observed in GR-null mice were not seen in GR dim mice suggesting that GR dimers are dispensable for skin development [14]. GR dim mice undergo a muscle atrophy similar to WT mice when treated with glucocorticoids despite attenuated induction of MuRF1, indicating that muscle atrophy is a dimer independent process [72]. Glucocorticoids induce osteoporosis in GR dim mice indicating this is a dimer independent process [47, 65]. GR dim mice were resistant to the therapeutic effects of glucocorticoid in an antigen induced arthritis

model of rheumatoid arthritis [46] indicating GR dimers are essential for this process. GR dim mice did not increase intestinal glucose uptake in response to glucocorticoids indicating this process is dependent on GR dimers [41]. GR dim mice were not immune to glucocorticoid-mediated changes in triglyceride metabolism in adipose, indicating this is a GR dimer independent process [65].

Another knockin mouse (MGI strain 3842978), of interest is a mutation that made GR more sensitive to ligand by mutation M610L in the LBD. These mice did not have a phenotype of glucocorticoid excess because their HPA axis was reset such that the circulating levels of glucocorticoids were lower than WT [73]. Muglia generated a knockin for a GFP-GR fusion protein allowing visualization of GR in vivo (MGI:3577992) [74].

Version 5.0: The Future of Mice with Altered GR Signaling

The advent of CRISPRs has made gene targeting more accessible given reduced cost, time and labor involved. First generation CRISPRs experiments were able to introduce double-strand breaks repaired in a stochastic manner involving non-homologous end-joining resulting in deletions and insertions [75]. Newer approaches allowed coinjection of a large single stranded donor oligonucleotide or double stranded DNA donor plasmid to induce targeted mutations via homologous recombination. Further advances have been made rapidly allowing the introduction of loxP sites to create floxed mice for conditional null alleles [76]. We predict this technology will lead to the creation of even more mice genetically modified in GR signaling. What mice will we see in the near future? Additional knock-in mice with point mutations leading to altered AF1 and AF2 function [77] would be insightful. In addition, point mutations for GR residues shown to be post-translationally modified would be informative as to the role of these post-translational modifications in vivo. In addition, there are a number of additional polymorphisms in GR that have been linked to human disease states including metabolic syndrome and depression [78] which could be informative in the function of GR in vivo. Finally, the ease of using CRISPRs to generate targeted germ line mutations could be the solution to a problem that has existed in the field of transcriptional regulation. We have access to whole genome transcriptional data and chromatin-immunoprecipitation data for regulation by glucocorticoids. It has been difficult to demonstrate that a given binding site is directly responsible for a given gene regulation event. Often this correlation is made by way of parsimony: Ockham's razor argues that the closest GR binding region to a gene regulated by GR is responsible for that gene's regulation. However, it is clear that there are a number of transcriptional events regulated from distal DNA elements. Therefore, gene targeting of a GRE would enable one to determine what genes are governed by the GRE. While it is not feasible to do this on a wide scale, and it is more feasible to do this in cell lines, we would not be surprised if we saw some GRE targeted CRISPRs in the near future. A handful of such mice might reveal rules to the logic of transcriptional regulation previously undecipherable. Candidates for such approaches would be GR primary target genes that are critical in a glucocorticoid-mediated phenotype with a well-defined GRE.

Non-GR Mutants Relevant to Glucocorticoid Signaling

We will not have space to describe all of the genetically altered mice related to glucocorticoid action, but a few notable mutations are described here. Given the regulation of glucocorticoid synthesis by the hypothalamic–pituitary–adrenal axis, it was of great interest to know the phenotype of CRH-null mice. CRH-null mice have reduced but detectable corticosterone levels. CRH-null animals born to heterozygous mothers survive, but CRH-null mice born to CRH-null mothers die at birth due to defects in lung maturation, similar to GR-null animals. If CRH-null mothers are given corticosterone in the drinking water, their CRH-null pups will survive. The peptide ACTH is encoded by the POMC gene which gives rise to several other peptides. Therefore, the phenotype of the POMC-null mice can not be attributed only to defects in ACTH. Mice lacking 7B2, a cofactor for proconvertase 2 (a protease that processes several endocrine protein hormones) display Cushing's disease, due to hypersecretion of ACTH [79]. Recently, a Cushingoid mouse was identified as part of an ENU mutagenesis screen [80]. The mutation was mapped to the CRH promoter (120 basepairs upstream of the transcriptional start site) in a caudal-type homeobox response element (CDXARE). The mutation leads to increased CRH expression and is recapitulated with a luciferase reporter. The phenotype of this mouse is similar to the CRH-Tg mouse as well as the mice with deletion of GR in tissues governing negative feedback in the hypothalamus including Sim1-cre and Nestin-Cre. Vale's group created a Cushingoid mouse by placing the CRH gene under a constitutive metallothionein promoter [81]. Interestingly, the investigators found that the transgene was only expressed in the hypothalamus and CRH levels could not be detected in the circulation. Yet, these mice had increased ACTH levels. The investigators postulated that a cryptic regulatory site within the coding region was responsible for this tissue specific transgene expression. While their peak corticosterone levels are not significantly different from WTs, their nadir levels are much higher, abrogating the circadian rhythmicity of corticosterone production similar to patients with endogenous glucocorticoid excess. CRH-Tg mice have increased adiposity, decreased bone density, decreased skin thickness and skin collagen production. Interestingly, CRH-Tg mice have large increases in adipose triglyceride futile cycling with increased rate of both lipolysis and reesterification [82]. CRH-Tg mice display age-dependent malabsorption that is due to a "transdifferentiation" of exocrine pancreas to express hepatocyte markers [83].

Dehydrogenase Mutants

To test the hypothesis that increased glucocorticoid signaling in adipose tissue plays a role in the etiology of metabolic syndrome Flier's group created an aP2-11-β-HSD1 transgenic mouse. Selective expression of this GC activating enzyme in adipose should raise tissue corticosterone levels in adipose. These mice displayed features of metabolic syndrome while on a chow diet [84]. Similarly, the converse

was shown, i.e. transgenic expression of 11-β-HSD2, the GC deactivating enzyme, protected mice from metabolic abnormalities when fed a high-fat diet [85]. A similar approach was used to modulate glucocorticoid levels in the bone [48].

GR Targets

A full description of genetic modifications to primary GR causative target genes is beyond the scope of this chapter, but we would like to highlight a few key observations. Given that glucocorticoid receptor alters transcription of hundreds to thousands of genes in most cell types it is amazing that elimination of a single GR target gene can affect the phenotype associated with glucocorticoid exposure. Furthermore, many glucocorticoid targets have functions distinct from their regulation by glucocorticoids. Glucocorticoid-mediated muscle atrophy occurs via transcriptional activation of atrogins such as MuRF1 and MAFbx (atrogin1). Deletion of MURF1 attenuates glucocorticoid-mediated muscle atrophy [86]. Similarly, deletion of the GR target Hes1 attenuates metabolic effects of glucocorticoids in the liver [87].

Summary and Looking Ahead

The last 25 years have seen a proliferation of our knowledge about glucocorticoid signaling. Essential to this has been the ability to probe the role of GR in vivo in mice using first anti-sense transgenics, the traditional knockouts, and then tissue specific knockouts. The tissue-specific GR KO mice have allowed a better understanding of which effects of glucocorticoids are cell autonomous and which are indirect. For example, muscle atrophy which could have been due to effects directly on muscle as well as indirectly through effects on liver (IGF1 secretion), the HPA axis (reduced sex steroids) appears to be largely cell autonomous. Similarly, a priori, effects on bone could be direct or indirect through reductions in other pituitary axes including GH-IGF1, hypothalamic–pituitary–gonadal, intestinal absorption of calcium, etc. Here, too effects seem to be direct on bone, and while the osteoblast appears to be critical, the osteoclast may play a role. Furthermore, some phenotypes are agreed upon by multiple investigators, such as the muscle specific KO mouse which has been made by three different investigators. Areas of discrepant data include the critical cell type in the lung responsible for the lethality of GR-null mice. The apparent discrepancy in phenotype of liver specific GR-null mice can be explained by the timing of activation of the cre lines used by the different investigators. Given the recent breakthrough in gene editing with CRISPRs, it is likely many more GR mutant mice will be generated and contribute further to our understanding of how GR signals. In addition CRISPRs may enable investigators that use other experimental models such as rat to also generate additional animals with gene modifications.

After detailing the multiple effects of the glucocorticoid receptor in the sections above, it is quite remarkable that the GR-null mouse makes it to birth and is not

embryonic lethal. In other words, although GR has been implicated in cell fate decisions, GR-null mice do not have an obvious loss of an essential cell type. The exceptions are the maintenance of adrenal chromaffin cells as adults and maintenance of some pancreatic cell types as mentioned above. Similarly, glucocorticoid excess alters some cell fate decisions with the best evidence in vivo being hepatic conversion of exocrine pancreas in CRH-Tg mice. Although the focus regarding neonatal mortality of GR-null mice has been on the lung, it is possible than even if this were rescued, GR-null mice might die due to effects on other tissues, such as skin or the heart. We predict the answers to these questions will come in the years ahead.

Additional Resources

In addition to this chapter, we suggest the reader use the following websites to assemble the most up to date information. Mouse genome informatics (http://www.informatics.jax.org/). This is a comprehensive listing of mouse genes with links to individual mice (i.e. separate listings for different strains created by different labs including knockout projects). There is a separate listing for the GR gene (NR3C1) as well as a listing for each mutant mouse. Each mutant mouse line is assigned a unique MGI number which is occasionally referenced in this chapter. There are links to references, as well as to many other data bases, including IMSR (international mouse strain resource, http://www.findmice.org/), a database listing where various mouse strains are held internationally.

Nuclear Receptor Signaling Atlas (NURSA, http://www.nursa.org/) contains valuable information on nuclear receptors and coregulators including links to MGI pages.

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