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Review

Search for a functional glucocorticoid receptor in the mammalian lens

Vanita Gupta [a,*], B.J. Wagner [b,c,d,e]

[a] Department of Pathology, Mount Sinai School of Medicine, One Gustav L. Levy Place, Box 1179, New York, NY 10029, USA
[b] Department of Biochemistry & Molecular Biology, UMDNJ, Newark, NJ, USA
[c] Department of Ophthalmology, UMDNJ, Newark, NJ, USA
[d] New Jersey Medical School, NJ, USA
[e] Graduate School of Biomedical Sciences, Newark, NJ, USA

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ABSTRACT

Prolonged glucocorticoid treatment of medical conditions such as rheumatoid arthritis or asthma can lead to the formation of a posterior subcapsular cataract as a negative side effect. Currently, the only treatment for this cataract is surgery because very little is known about the mechanism of glucocorticoid action in the mammalian lens. Understanding of a lens glucocorticoid response is essential for the treatment and prevention of a steroid induced cataract. It has been suggested that glucocorticoids exert their effects on the lens indirectly, non-specifically, or through non-classical mechanisms. While these modes of action may contribute to the formation of glucocorticoid induced posterior subcapsular cataract, the finding of a classical, specific, functional lens glucocorticoid receptor suggests that glucocorticoids target lens epithelial cells directly, specifically, and similar to what has been observed in other cells types. This review explores the discovery of the glucocorticoid receptor in humans lens epithelial cells and the lens specific glucocorticoid response. The distinct changes in lens epithelial cell signaling pathways (MAPK and PI3K-AKT) suggest that glucocorticoids modulate several cellular functions and may explain why a lens glucocorticoid response has been difficult to elucidate.

1. Introduction

Glucocorticoids, or corticosteroids, are a major subclass of steroid hormones involved in physiological processes including metabolic, cardiovascular, immune, and behavioral functions. Administration of synthetic glucocorticoids (GC), as immunosuppressive and anti-inflammatory agents, is a commonly employed form of treatment for a large group of frequently encountered diseases and medical conditions from rheumatoid arthritis and asthma to organ transplants and chemotherapy. The incidence of clinical conditions requiring glucocorticoid therapy is expected to increase with age and thus elucidation of GC action and all possible side effects associated with this type of therapy is important. Side effects occur with prolonged GC use or when higher than physiological doses are administered for immunosuppresion (Kimberly, 1991). It is well documented that a side effect of prolonged GC use is steroid induced cataract (Black et al., 1960; Harve, 1965; Williamson et al., 1969; Urban and Cotlier, 1986; Kaye et al., 1993; Thompson and Lippman, 1974).

Cataracts are reported to occur in 11–38% of patients and can occur as early as after 6 months of treatment, but typically, lenticular changes are not seen until steroids have been administered for prolonged periods (Giles et al., 1962; Frangie and Leibowitz, 1993). Discontinuing GC treatment can halt cataract formation but does not always reverse the damage done, though reversal is possible in some cases (Seth and Aggarwal, 2004; Ansell, 1991). If opacification is great and obscures vision, cataract can only be corrected with surgery. Cataract extraction is expensive and there are high risks of complications that lead to subsequent blindness (Weintraub et al., 2002).

The mechanism and pathogenesis of a steroid induced cataract are not known. Several mechanisms of cataractogenesis have been proposed, including binding of GCs to the hepatic glucocorticoid receptor (GR) (Watanabe et al., 2000; Nishigori et al., 1986; Pescosolido et al., 2001; Dickerson et al., 1997; Murakami et al., 1996), non-specific GC binding in the lens (Jobling and Augusteyn, 2001b; Bucala et al., 1982; Manabe et al., 1984), and binding to a membrane receptor (Zhu et al., 2001). GCs have been demonstrated in the aqueous humor (Knisely et al., 1994; Obenberger et al., 1971) and recently, the classic active GR was found in lens epithelial cells (Gupta and Wagner, 2003) demonstrating a direct and specific mechanism of action. The identification of a classic, specific and functional lens GR sheds some light on GC action in the lens and the

* Corresponding author.
E-mail address: vanitagupta@gmail.com (V. Gupta).

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difficulties in studying a steroid induced posterior subcapsular cataract. This review will focus on the discovery of the GR and demonstration of changes in signaling pathways due to specific lens GR activation. For a review of the etiology of a steroid cataract, including non-GR mechanisms, see the recent review by James (2007). The understanding of lens GC action will have an enormous impact on the clinical use of glucocorticoids because it can lead to the prevention of cataract formation, a common deleterious side effect of glucocorticoid action.

2. Posterior subcapsular cataract

Black et al. (1960) first noted that corticosteroid therapy could result in the formation of a posterior subcapsular cataract in 39% of patients (Giles et al., 1962; Frangie and Leibowitz, 1993). The steroid induced opacity in a posterior subcapsular cataract (PSC) occurs bilaterally and is clearly differentiated from the more common senile cataract and some other forms of posterior subcapsular cataract (Giles et al., 1962; Ogelsby et al., 1961). Preparations of equatorial and posterior PSC capsules show abnormalities with nucleated epithelial cells migrating from the equatorial region to the posterior region of the lens, which is normally devoid of epithelial cells. These migratory cells have been thought to be proliferative and the finding of bilateral lesions, that correlated with dose as well as duration of treatment, only in patients receiving treatment, as well as the finding of nucleated epithelial cells in the posterior region (Black et al., 1960; Harve, 1965; Williamson et al., 1969; Urban and Cotlier, 1986; Kaye et al., 1993; Thompson and Lippman, 1974; Greiner and Chylack, 1979; Eshagian, 1982) suggests GCs may play a direct role in lens cell function. In contrast to proliferative or migratory lens epithelial cells, primary cultures of human lens epithelial cells (hLECs) grown in the presence of the synthetic steroid prednisolone demonstrated a decreased growth rate and the number of cells declined after 20 days presumably due to cell death (Jacob et al., 1987). This may be related to observations of abnormalities in cell morphology, size, and density noted in posterior subcapsular cataracts (Karim et al., 1987). Specifically, an increase in cell volume, disturbances in the hexagonal array, and vacuoles, with large gaps between cells, were observed in patients or explant PSC from patients receiving steroid therapy for 1–3 years (Black et al., 1960; Jacob et al., 1987).

GCs, both endogenous and after steroid therapy, have been found in the aqueous humor (Knisely et al., 1994; Obenberger et al., 1971) and the abnormalities suggest that GCs may disturb normal lens cells function, particularly proliferation, differentiation, migration, or apoptosis. Furthermore, GCs have been shown to be involved in proliferation, differentiation, migration, apoptosis, and survival of other cell types (Kawamura et al., 1998; Gao et al., 2002; Wang et al., 2003b; Bomberger and Haar, 1992).

3. Glucocorticoids act via the glucocorticoid receptor

The name glucocorticoid derives from early observations that these hormones were involved in glucose metabolism. Glucocorticoids, crucial for the proper function of the body, originate in the adrenal cortex and facilitate the metabolism of carbohydrates, fat, and protein, to increase gluconeogenesis and provide fuel to the body in times of stress (O'Malley, 1971). GCs also play a role in normal immune responses because they have potent anti-inflammatory and immunosuppressive properties, but this is particularly evident when they are administered at pharmacologic doses (Clark, 1968). As a consequence, they are widely used medically, especially for suppression of allergic, inflammatory, and autoimmune diseases and to suppress rejection of transplanted organs. GCs used clinically include the naturally occurring GCs, cortisone and cortisol, and synthetic derivatives, such as dexamethasone and prednisolone (Fuenfer et al., 1975). In conditions that require the short term use of GCs, side effects do not often pose a problem. Side effects are more often seen in long term therapy or when given in high doses (Thomas, 1984).

At a cellular level, GCs exert their biological effects by binding to a specific intracellular receptor, the glucocorticoid receptor (GR) (Baxter and Rousseau, 1979). The GR belongs to a phylogenetically conserved superfamily of nuclear hormone receptors that includes receptors for mineralocorticoids, androgens, estrogens, progestins, vitamin D, retinoic acid, thyroid receptors and a growing number of orphan receptors. Nuclear hormone receptors act as ligand inducible transcription factors by directly binding to hormone response elements as monomers, homodimers and heterodimers.

The non-liganded hypophosphorylated GR is inactive and resides in the cytoplasm as a multi-protein complex involving heat shock proteins (Bertorelli et al., 1998; Orti et al., 1993). GCs are lypophilic molecules that readily cross the cell membrane by passive diffusion and bind to the GR (Orth and Kovacs, 1998). Agonist ligand binding activates the receptor through inducing a conformational change which results in dissociation of the heat shock proteins and hyperphosphorylation of the receptor (Rowan and Ip, 1995). The conformational change gives the receptor–hormone complex the ability to bind DNA and modulate the expression of target genes (Burnstein et al., 1991). The liganded receptor translocates to the nucleus, dimerizes, and binds to a *cis*-acting element, the glucocorticoid response element (GRE), typically present in the 5′ flanking region of the target gene (Slater et al., 1986).

The active receptor affects transcription through several mechanisms (Fig. 1). The GC response can be divided into a primary and a secondary response. A primary response involves direct GC–GR binding to a GRE located on that target gene to directly modulate transcription. A secondary response involves the product of the primary response modulating the expression of another gene (Shepard et al., 2001). GCs also affect transcription by directly binding to a transcription factor, such as c-Jun, to prevent c-Jun from dimerizing with its essential binding partner or by binding to a dimerized transcription factor to prevent its action with the basal transcription machinery. The binding of the GR to a transcription factor prevents the factor from modulating the expression of genes (Saklatvala, 2002). Regardless of the mechanism, the presence of the GR is necessary for GCs to specifically exert their effects in a cell.

4. Glucocorticoids and the mammalian lens

Although GCs induce posterior subcapsular cataracts after both topical and systemic administration (Giles et al., 1962; Becker, 1964) it is not known if this effect is due to direct action on LECs, binding of GC to a lens GR, or through a metabolic effect as a result of GC binding to a GR at another site. Several groups have hypothesized mechanisms of GC action.

Studies in chick embryos have suggested that GCs act on the lens indirectly. It has been hypothesized that GCs act indirectly by binding to classical GRs in the liver (Watanabe et al., 2000; Nishigori et al., 1986), resulting in an increase of blood lipid peroxides which travel to the aqueous humor and results in the depletion of lens glutathione. The oxidative stress caused by glutathione depletion is thought to result in the formation of a steroid induced cataract in chick embryos (Watanabe et al., 2000; Nishigori et al., 1986; Pescosolido et al., 2001). However, GC treatment of embryonic chick lenses, which do not contain a GR, results in nuclear opacities, not posterior subcapsular cataracts, suggesting a different mechanism of opacification in the mammalian lens.

Jobling et al. (2001a)(b) demonstrated the non-specific binding of GC to α-crystallin in the bovine lens epithelium. Another hypothesis of non-specific GC action is related to α-crystallin binding which involves the covalent addition of steroids to lens proteins

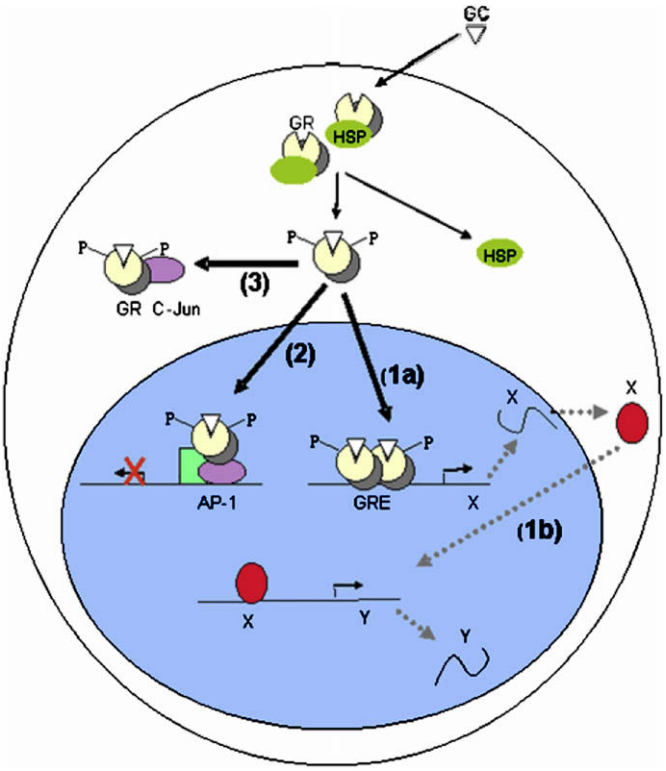

Fig. 1. Glucocorticoid action. Glucocorticoid receptors reside primarily in the cytoplasm bound to a multi-protein complex including heat shock proteins (HSPs). GCs passively diffuse through the cell membrane and activate the GR by causing a conformational change in the GR which results in the dissociation of the multi-protein complex. The activated GR translocates to the nucleus and modulates transcription by homodimerizing and binding to a GRE on a target gene (1a). The product of the target gene can in turn act as a transcription factor to modulate the transcription of other genes (1b) resulting in a secondary GC effect. The GR can also act by preventing transcription factors, such as AP-1, from interacting with the basal transcription machinery (2) or sequestering factors by preventing them from dimerizing with their partners (3).

(Bucala et al., 1982; Manabe et al., 1984). This destabilizes protein conformation to allow the oxidation and cross linking of protein thiol groups.

Also, a membrane steroid binding protein that specifically binds GC was recently identified in bovine lens epithelial cells, but its mRNA and protein sequence differ from the classical glucocorticoid receptor (Zhu et al., 2001). This membrane protein may play a role in the formation of a steroid induced cataract but must act by rapid non-genomic actions.

While these mechanisms of action may be involved in PSC, they fail to address the most common mechanism of GC action: through a classic GR. Southren et al. (1978) demonstrated that the bovine lens epithelium contained a glucocorticoid binding protein that exhibited the characteristics of a classical receptor. Furthermore, autoradiographic studies demonstrated that a tritiated synthetic steroid, Dex, localized over the nucleus in bovine lens epithelial cells which again is a characteristic of a GR (Wenk et al., 1982). Stokes et al. (2000), and later confirmed by Suzuki et al. (2001), identified the GR in the human and rat lens epithelium through immunohistochemistry and in situ hybridization.

Despite these findings, questions remained. Results from competition studies with synthetic steroids in the lens differed from competition studies done with a classical GR in the liver (Wenk et al., 1982). It is interesting to note, however, that the results from lens competition studies were similar to results from GR competition studies in the iris and ciliary body of the mammalian eye suggesting, perhaps, tissue or organ specific responses. Yet, Jobling et al. (2001a) demonstrated the binding of GC to the bovine lens epithelium, but the binding was non-specific and did not exhibit the characteristics of a receptor. Also, researchers have failed to identify changes in gene expression or in DNA and protein metabolism in lens cells treated with GCs (Kawamura et al., 1998; van Venrooij et al., 1974). These contradictory results left many questions about the presence of a lens GR and the ability of GC to target the mammalian lens epithelium specifically.

5. The lens contains a classic functional GR

When the human GR was cloned in 1985, two highly homologous cDNA clones, α and β, that differed in the C-terminal domain, were identified (Hollenberg et al., 1995; Oakley et al., 1997). The GRα is the predominantly expressed isoform, functions as a ligand dependent transcription factor and its presence is necessary for both natural and synthetic GCs to elicit a biological response. The GRβ has a widespread tissue distribution but is unable to bind hormone, does not activate glucocorticoid responsive promoters and acts as a dominant negative repressor inhibiting GRα transactivation of target genes (Oakley et al., 1997; Giguere et al., 1986; Bamberger et al., 1995). The contradictory findings in the mammalian lens could be explained by lack of GRα expression or an over expression of the dominant negative GRβ. However, in 2003, the presence of the lens GRα was confirmed through the identification of its mRNA by reverse transcription PCR (Gupta and Wagner, 2003; James et al., 2003). The sequence was 98–100% identical to previously published sequences of the GR, in αTN4 and HLE B-3 immortalized mouse and human, respectively, lens epithelial cell lines and in freshly isolated mouse and human lens epithelia (Gupta and Wagner, 2003). The GRα isoform was reported to be the predominant isoform with the ratio of GRα to GRβ expression in human lens cells consistent with functional GR in other cell types (Hollenberg et al., 1985; Gupta and Wagner, 2003; Pujols et al., 2002).

In addition to finding GR mRNA expression, inactive and active GR protein was confirmed in the HLE B-3 and αTN4 cell lines and freshly isolated human lens epithelium (Gupta et al., 2005; Gupta et al., 2007). The GR undergoes hyper-phosphorylation at multiple serine residues upon hormone binding, of which three in the N-terminal region, S203, S211, S226, have been reported to be involved in transcriptional regulation (Lee et al., 2005). Phosphorylation of the GR was identified at all three serine residues in HLE B-3 cells treated with the synthetic GC, dexamethasone (Dex). This phosphorylation was inhibited by co-treatment with RU-486 (Gupta et al., 2007), a GR competitive inhibitor (Baulieu, 1991) that prevents agonist dependent phosphorylation (Orti et al., 1989).

The GR binds to *cis*-acting glucocorticoid response elements (GRE) located in promoters of target genes to modulate transcription (Slater et al., 1986). The lens GR was shown to be functionally active, bind to a classical GRE and to modulate the expression of a reporter vector in cells treated with a single treatment of physiological and pharmacological concentrations of GC over 72 h (Gupta and Wagner, 2003; Gupta et al., 2007). However, the GR shares sequence homology in the ligand binding and DNA binding domains with the mineralocorticoid receptor (MR) (Aranda and Pascual, 2001; Lombes et al., 1993). The MR is not only capable of binding to aldosterone with high affinity, but also binds the endogenous glucocorticoid, cortisol, and the synthetic steroid, Dex, with greater affinity than the GR (Lombes et al., 1993; Arriza et al., 1987; Rupprecht et al., 1993). The human lens epithelium was reported to express both the GR and MR (Stokes et al., 2000; Suzuki et al., 2001), but it was confirmed that Dex induced changes in reporter gene expression in lens cells are due to the binding of the activated GR, not MR, to the GREs of target genes (Gupta et al., 2007). Receptor specificity was determined by the GR antagonist,

RU-486 and the MR antagonist, spironolactone. The GR antagonist, RU-486, but not spironolactone, inhibited Dex induced increase in reporter activity. This conclusively demonstrated that the mammalian lens contained a classical functionally active GR, but still a lens specific lens GR response had not been identified.

6. GCs do not induce an acute change in lens cell function

GCs are known to elicit divergent biological outcomes modulated by the active GR (Kawamura et al., 1998; Gao et al., 2002; Wang et al., 2003b; Bomberger and Haar, 1971) and lens GC responses have been difficult to characterize. It has been hypothesized that the finding of a steroid cataract with nucleated epithelial cells in the posterior region of the lens could be due to increased cell proliferation (Karim et al., 1987; Jacob et al., 1987). GCs have also been reported to play a role in apoptosis and differentiation of other cell types, including T lymphocytes and osteoblasts (Amsterdam and Sasson, 2002; Distelhorst, 2002). Lens epithelial cells undergo an apoptotic like process during differentiation. Differentiating lens cells share many morphological and biochemical changes with cells undergoing programmed cell death, which involves the degradation of nuclei (Thompson and Lippman, 1974). It has been hypothesized that posterior subcapsular cataract may be the result of stalled differentiation in the equatorial region of the lens and the migration of the dysplastic cells to the posterior region of the lens (Eshaghian and Streeten, 1980). However, Dex, which has been shown to be cell type specific (Reichardt et al., 2001; Chapman et al., 1997), did not induce changes in cell proliferation or apoptosis in HLE B-3 cells (Gupta et al., 2007). Steroid induced cataract is observed after at least 6 months of prolonged GC treatment (Black et al., 1960; Harve, 1965; Williamson et al., 1969; Urban and Cotlier, 1986; Kaye et al., 1993; Thompson and Lippman, 1974). It is possible that Dex plays a role in LEC proliferation or apoptosis, but treatment alone may not be sufficient to yield an observable response or perhaps prolonged GC treatment is necessary to observe a physiological response in lens epithelial cells.

7. GC treatment results in modulation of gene expression

GCs have been proposed to affect lens epithelial cells through a variety of mechanisms, but the identification of a transcriptionally active intracellular GR suggests that GCs may be modulating the transcription of target genes. Although GC effects on lens epithelial cells, such as changes in protein expression (Lyu et al., 2003), the formation of a cataract, and changes in gene expression (James et al., 2005) have only been observed after greater than 24 h of treatment, recent findings demonstrate GCs can play a role in gene expression as early as 2 h (Gupta et al., 2005). Monitoring the expression levels of thousands of genes through microarray technology led to the identification of GC induced changes in lens gene expression as early as 4 h (Gupta et al., 2005) and as late as 48 h (James et al., 2005). Furthermore, several of the transcripts identified, including glucocorticoid induced leucine zipper (GILZ), map kinase phosphase-1 (MKP-1), and plasminogen activator inhibitor (PAI), contain putative GREs in the promoter regions suggesting that the modulation of these genes is due to direct binding of the activated GR to the GRE of these promoters in hLECs (Gupta et al., 2005).

In a primary GC response, no new protein synthesis is needed as the activated GR binds directly to a GRE to activate gene transcription. A secondary response involves the elapse of time during which the products of the primary response act as positive and negative transcription factors or co-factors. Ninety-three transcripts were observed to be upregulated and 43 transcripts downregulated in LECs treated with GC for 4 h (Gupta et al., 2005). It is interesting to note that after 16 h of treatment, 30 transcripts were upregulated while 56 were downregulated. It is possible that the large number of downregulated transcripts at 16 h could be due to a secondary glucocorticoid response with the expression of co-repressors that were upregulated at 4 h (Gupta et al., 2005). However, by 24 h, it appears that the number of up regulated and down regulated transcripts is nearly equal but by 48 h, the number of up regulated genes is markedly increased (James et al., 2005). GCs appear to be modulating genes differently over time.

Although genes were modulated, a specific lens physiological cell response to Dex treatment was not identified through cluster analysis and gene ontology mining (Gupta et al., 2005). Dex treatment did not result in coordinate modulation of a group of genes that work together in a specific cellular function. Instead, GCs appeared to have diverse effects on the cells, affecting many biological and molecular functions. Although the modulated genes may not affect a specific cell function, such as proliferation or apoptosis, further analysis suggested that they may instead be involved in signaling pathways that target different cell functions. Changes in lens cell signaling that affect a wide array of cellular processes could cancel each other to produce no short term net effect. Perhaps this can account for the difficulty researchers have had in identifying a functional response to glucocorticoid treatment in lens epithelial cells.

8. MAPK and PI3K/AKT and glucocorticoid signaling

The mitogen activated protein kinases (MAPKs) are proline directed serine/threonine kinases regulated by a phosphorylation cascade and are critical for the transduction of diverse extracellular signals to regulate cell proliferation, death, survival, differentiation and motility (Seger and Krebs, 1995). The MAPKs are grouped into three subfamilies, extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and p38s. ERKs are associated with survival and are activated in response to mitogens and growth factors. JNK and p38 are associated with apoptosis and are activated in response to cell stress. Regulation of MAPK activity can be controlled by kinases that activate the pathway and phosphatases which inactivate ERK 1/2, JNK, and p38. The dynamic balance between ERK, p38, and JNK and their regulators determine whether the cells undergo proliferation, differentiation, or apoptosis (Theodosiou and Ashworth, 2002).

The PI3K/AKT pathway is involved in cellular responses such as cell survival, cell proliferation, cell growth, and transformation. AKT, also known as protein kinase B, is a serine/threonine protein kinase that prevents apoptosis and is activated by phosphorylation through upstream kinases in the PI3K cascade. Similar to MAPK, the activity of PI3K/AKT is negatively regulated by phosphatases (Chang et al., 2003). MAPK and PI3K/AKT pathways have been reported to cross talk with each other in lens epithelial cells (Balendran et al., 1999; Zatechka and Lou, 2002). In other cell types, GCs have been reported to crosstalk with the MAPK pathway through modulation of MAPK regulators, post-transcriptional regulation of similar genes, and regulation of cellular functions (Clark and Lasa, 2004). GCs have also been reported to inhibit the activation of PI3K/AKT via the GR (Andrade et al., 2004). Glucocorticoids, MAPKs, and PI3K/AKT are involved in cell proliferation, growth, apoptosis, differentiation, motility, transformation and survival (Kawamura et al., 1998; Gao et al., 2002; Wang et al., 2003b; Bomberger and Haar, 1992; Seger and Krebs, 1995; Chang et al., 2003) all of which have been implicated in steroid induced cataract.

Lens GC modulated genes appear to be regulators or downstream targets of the MAPK and PI3K/AKT pathways pathway. The glucocorticoid induced leucine zipper (GILZ), also known as delta

sleep inducing peptide-like immunoreactor (DSIP), was significantly upregulated by GC treatment in hLECs and its expression was sustained over 48 h (Gupta et al., 2005; James et al., 2005). GILZ directly interacts with RAF-1, prevents RAF-1 phosphorylation and activation and subsequently affects the RAF- MAPK pathway by preventing the phosphorylation and activation of ERK 1/2 (Ayroldi et al., 2002). GILZ also interacts with other components of the MAPK pathway as well as their targets, c-Fos and c-Jun (Mittelstadt and Ashwell, 2001).

Dual specificity phosphatase-1, also known as MKP-1, was found to be upregulated after 16, 24, and 48 h of Dex treatment (Gupta et al., 2005; James et al., 2005). MKP-1 is a protein tyrosine phosphatase which dephosphorylates ERK, JNK, and p38 (Theodosiou and Ashworth, 2002). MKP-1 was identified to be transcriptionally induced by GCs with simultaneous inactivation of ERK in mast and osteoblast cells (Engelbrecht et al., 2003; Wu and Bennett, 2005a). This up-regulation is both GR and protein synthesis dependent and makes use of functional GREs in the promoter region of the MKP-1 gene (Kassel et al., 2001; Noguchi et al., 1993).

Several other genes, modulated by GC treatment in HLE B-3 cells have been shown to be involved with both the PI3K/AKT and MAPK pathways suggesting GC modulation of signaling pathways (Gupta et al., 2005, 2007; James et al., 2005). It is also interesting to note, in trying to identify a GC lens response, that high dose prolonged Dex treatment of rat lens resulted in a decrease in the protein expression of E-cadherin and N-cadherin without changes in mRNA expression (Lyu et al., 2003). Both the MAPK and PI3K/AKT pathways have been reported to be involved with E-cadherin expression and activity (Laprise et al., 2004).

9. GCs treatment results in a change in lens cell signaling

Analysis of microarray data suggested that GCs affect many cellular functions, such as proliferation, differentiation, apoptosis, survival, or migration, through modulation of the components and regulators of the PI3K/AKT and MAPK pathways (Gupta et al., 2005; James et al., 2005) (Fig. 2). This was confirmed by RNA and protein analysis in HLE B-3 cells and primary cultures of human lens epithelial cells (Gupta et al., 2007). GCs induced an increase in GILZ and MKP-1 expression and a decrease of phospho-RAF, but not total RAF expression, which preceded the decrease in phospho-ERK (Fig. 2) (Gupta et al., 2007). A significant decrease in phospho-p38 was detected as well, but not in phospho-JNK (Gupta et al., 2007). This is not surprising since Dex has been reported to activate or inhibit the MAPKs differentially depending on the cell type and conditions (Noguchi et al., 1993; Gonzalez et al., 1999; Zhang et al., 2000; Bazuine et al., 2004; Wu et al., 2005b). In addition, Dex treatment of HLE B-3 demonstrated a significant decrease in phospho-AKT without a change in total AKT (Fig. 2) demonstrating modulation of the PI3K pathway. This is similar to what is seen in

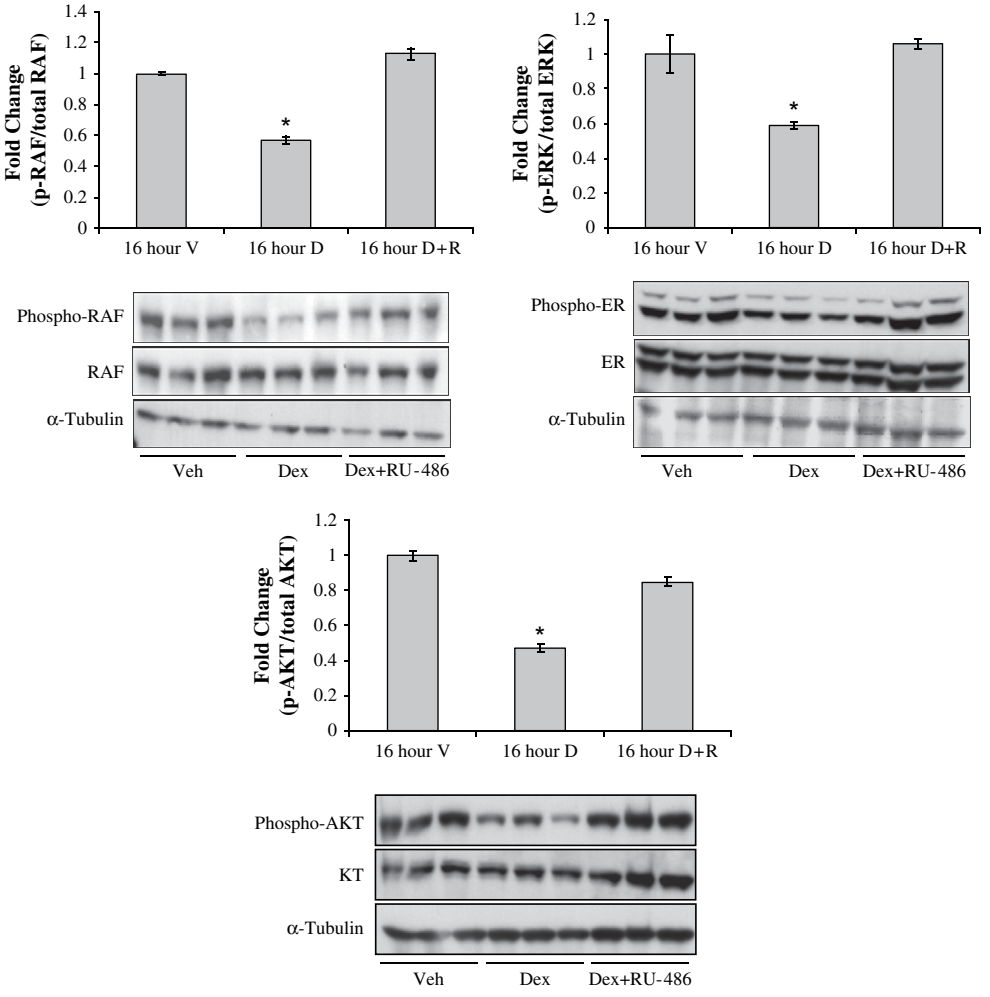

Fig. 2. Glucocorticoids reduce MAPK and AKT phosphorylation in HLE B-3 cells. HLE B-3 cells treated with vehicle, Dex, or Dex + RU-486 for 16 h in triplicate demonstrated decreased phosphorylation of RAF, ERK and AKT with Dex treatment compared to vehicle. The decrease in phosphorylation was inhibited by co-treatment with RU-486, the GR antagonist. No change was observed in total RAF, ERK, or AKT expression with treatment. From Gupta et al. (2007) with permission of the copyright holder of this figure, the Association for Research in Vision and Ophthalmology.

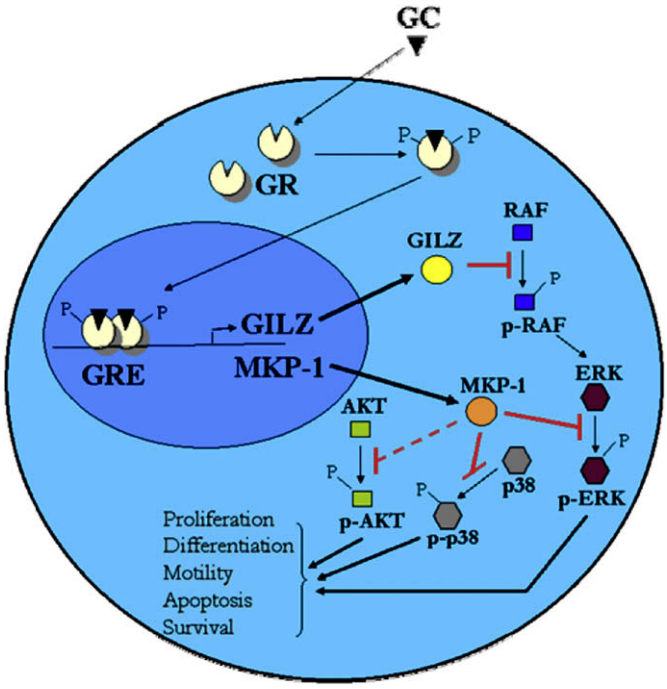

Fig. 3. Glucocorticoid treatment of hLEC results in activation of the GR, modulation of gene expression, and modulation of the MAPK and PI3K/AKT pathways. The MAPK and PI3K/AKT pathways play a role in cell proliferation, cell differentiation, cell motility and cell death, all of which have been implicated in posterior subcapsular cataract formation. Glucocorticoids are known to play a role in these same cell processes. GCs may be involved in lens cell function and cataractogenesis through the inhibition of these signaling pathways which includes upregulation of GILZ and MKP. GILZ has been reported to inhibit RAF phosphorylation. MKP-1 has been reported to dephosphosphorylate p38 and ERK, and possibly AKT.

other cell types. GC treatment of SCC cells resulted in decreased expression of phospho-ERK and phospho-AKT that was GR dependent (Bernardi et al., 2001) and MKP-1 expression was observed with decreased phospho-ERK and phospho-AKT in hepG2 and MCF-7 cells (Wu et al., 2005b; Zhou et al., 2003).

Since the MAPK and PI3K/AKT pathways have been reported to play roles in important cell stress responses, similar cellular functions, and cross talk with each other in many cell types including lens epithelial cells (Zatechka and Lou, 2002; Clark and Lasa, 2004; Chan, 2005) it is possible that prolonged modulation of these pathways could lead to abnormal lens epithelial cell proliferation, differentiation, motility, survival, or apoptosis, all of which have been implicated in the formation of a steroid induced cataract. This is supported by the fact that regulators of the pathways, as well as their downstream targets, were reported to be modulated as early as 4 h and as late as 48 h after Dex treatment of LECs demonstrating prolonged modulation with a single GC treatment (Gupta et al., 2005; James et al., 2005).

There are reports suggesting the RAF/ERK pathway acts upstream of the PI3K/AKT pathway (Hideshima et al., 2001) and reports of the PI3K/AKT pathway acting upstream of the RAF/ERK pathway (Meier et al., 2005). The crosstalk observed in LECs appears to be redundant at several levels of the signaling pathways in the early GC lens response. For example, RAF has been found to be a target of PI3K (Zatechka et al., 2003; Chaudhary et al., 2000), so the decrease in both phospho-AKT and phospho-RAF-1 at 4 h Dex treatment (Gupta et al., 2007) hints that one pathway may not be upstream of the other. It has been suggested that these signals are not transmitted in a linear fashion, but instead through a combination of signals that converge on a final target or cellular function (Zatechka and Lou, 2002).

The activity of the MAPK and PI3K/AKT pathways depends on the dynamic balance between its activators and inhibitors. It is interesting to note that AKT and ERK are survival factors known to upregulate antiapoptotic factors suggesting that GC induced inhibition of AKT and ERK phosphorylation inhibits lens cell survival. However, p38 is associated with promotion of apoptosis (Lee et al., 2003) and decreased phospho-p38 was seen as well (Gupta et al., 2007). It is possible that inhibition of cell apoptosis, through inactivation of p38, is a mechanism to compensate the inhibition of cell survival, through inactivation of ERK and AKT maintaining a steady state response.

JNK also plays a role in apoptosis (Seger and Krebs, 1995) but no changes in JNK were observed (Gupta et al., 2007). It is interesting to note that microarray analysis revealed increased expression of GADD45, which positively regulates JNK. This may balance the expression of MKP-1, which inactivates JNK. This demonstrates the complexity of the GC response and may account for the inability of researchers to identify early physiological responses, such as changes in proliferation or apoptosis, with an acute Dex treatment (Gupta et al., 2007). It is interesting to note that recent studies examining the effects of Dex in rat lens explant models of PCO demonstrated changes in lens epithelial cell behavior at a physiological concentration of Dex (Mansfield et al., 2004; Symonds et al., 2006). These effects, however, were observed after the explants were treated with TGF-β and/or FGF-2. Other studies of rat lens explants treated with Dex alone suggest that Dex can induce similar changes in lens cell behavior, but after chronic high dose treatments (Lyu et al., 2003). In general, cellular changes have only been identified after high dose prolonged GC treatment (Black et al., 1960; Harve, 1965; Williamson et al., 1969; Urban and Cotlier, 1986; Kaye et al., 1993; Thompson and Lippman, 1974; Giles et al., 1962; Frangie and Leibowitz, 1993; Jacob et al., 1987). Although studies looking at the effect of GCs on LEC function yielded negative or inconclusive results in an observable physiological response, it is apparent that GCs elicit an early response through the modulation of the PI3K/AKT and MAPK pathways, which may affect a cellular function over time.

The MAPK and PI3K/AKT pathway have been implicated in other types of cataracts and mechanisms of lens opacification. Altered MAPK activity has been shown to be sufficient to result in cataract formation (Gong et al., 2001). Recent evidence has demonstrated that MAPKs are important for regulating LEC proliferation (Choi et al., 2004), apoptosis (Long et al., 2004), migration (Wang et al., 2003a) and cataractogenesis (Bomser, 2002). Modulation, through activation or inhibition, of any of these cellular functions can lead to abnormal lens cell function. Modulation of the PI3K pathway has also been implicated in the formation of a cataract (Chandrasekher and Sailaja, 2004) and has been demonstrated to act upstream of the MAPK pathway in LEC proliferation (Choi et al., 2004). Prolonged modulation of the MAPK and PI3K pathways could lead to the formation of a steroid induced cataract (Fig. 3).

10. Speculation on the mechanism by which prolonged exposure to steroids leads to cataractogenesis

In vitro Dex treatment of lens cells results in modulation of signaling pathways without a resultant observable physiological change. GC treatment resulted in changes in proliferation and migration after high-dose prolonged treatment times (>7 days) in primary cultures of human LECs (Jacob et al., 1987) or rat organ explants (Lyu et al., 2003). In addition, cataract is only observed after greater than 6 months of treatment (Frangie and Leibowitz, 1993). Although modulated gene expression was observed after an acute treatment (<24 h), neither low nor high-dose Dex treatment affected HLE B-3 proliferation or apoptosis (Gupta et al., 2007).

This suggests that lens cells are able to maintain homeostasis after an acute treatment. Furthermore, GC treatment alone may not be sufficient to yield a physiological response with a short treatment time. The final physiological response may be due to the cumulative response of the treatment and the other effectors, hormones, and growth factors present in the environment. The lens epithelium is divided into three regions: central, peripheral, and equatorial. The lens is surrounded by the aqueous humor on the anterior face with a gradient of growth factors and effectors present. The concentration gradient influences the epithelial cells, in the specific regions, to respond differently. The factors present in the peripheral region result in proliferation of LECs while those present in the equatorial region result in differentiation of LECs. GC treatment may result in a modulation of pathways that may aid LECs in the anterior region to proliferate, or LECs in the equatorial region to migrate or differentiate. Or else, the GC modulation of these pathways may antagonize these normal cell functions but the net effect of a short term GC treatment results in no change in physiological cell function due to the presence or response of other growth factors and effectors.

However, physiological responses observed after prolonged treatments suggest that chronic GR activation can lead to phenotypic changes. Perhaps long term GC treatment leads to more dramatic changes in gene expression, which result in prolonged modulation of these important signaling pathways leading to aberrant cell function. Prolonged modulation of the MAPK or PI3K/AKT pathways may lead to increased proliferation, decreased differentiation, increased migration, decreased apoptosis, or increased cell survival, all of which have been implicated in the finding of nucleated epithelial cells in the posterior region of the lens resulting in a posterior subcapsular cataract.

Prolonged modulation of these pathways could be due to the prolonged expression of MKP-1 or a tissue specific regulation of the GR. MKP-1 has a short half-life and is rapidly degraded by the ubiquitin–proteasome pathway (Kassel et al., 2001). Sustained MKP-1 expression is necessary for decreased phospho-MAPK expression. In NIH-3T3 cells, GCs upregulate MKP-1 expression, but do not attenuate its degradation by the proteasome, and as a result, do not affect phospho-ERK expression (Kassel et al., 2001). However, in contrast to observations in NIH-3T3 cells, GC treatment of LECs has demonstrated a greater than 4-fold increase in MKP-1 protein as late as 16 h with a decrease in phospho-ERK and phosho-p38 expression (Gupta et al., 2005; Gupta et al., 2007). This could be due to decreased degradation of MKP-1 by the ubiquitin– proteasome pathway. Interestingly, co-culture with RU-486 demonstrated that these changes are dependent on the GR, which is also subject to degradation by the ubiquitin–proteasome pathway (Ismaili and Garabedian, 2004). Sustained GR signaling was observed as late as 72 h suggesting decreased degradation of the GR (Gupta et al., 2005). It is possible that both MKP-1 and GR protein expression are stabilized by decreased proteasome activity.

It is interesting to note that steroid induced cataract appears to be due to exogenous steroid treatment. Cushing's syndrome is characterized by high levels of endogenous glucocorticoids, but patients do not develop a steroid induced cataract (Bouzas et al., 1993). Studies examining the GR in patients with Cushing's syndrome have suggested that it retains its high affinity for GCs but the number of GC binding sites is reduced, suggesting a normal downregulation of the receptor (Lamberts, 2002). Perhaps exogenous steroid therapy results in impairment of the ubiquitin–proteasome pathway or abnormal GR downregulation.

Regulation of the GR in terms of its isoforms and its targets is important to understand steroid cataract formation. A translational variant of the GRα isoform was identified due to different translational start sites (Yudt and Cidlowski, 2001; Russcher et al., 2005). The two isoforms, GRαA and GRαB, differ on the N-terminal domain. The shorter B isoform was found to be more responsive to GCs and more sensitive to degradation by the proteasome than the longer A isoform. Expression of the A isoform of the GRα in the lens could help explain the prolonged GC response over 72 h.

It has been difficult to use an animal model to understand the formation of a steroid cataract in humans. The chick is the most common model currently used to study a lens GC response, but as previously mentioned, the chick eye lacks a GR and does not result in cataracts similar to those observed in humans. In 1985, a posterior subcapsular cataract was observed in rabbits treated with GC for 72 h but the utility of the model remains unconfirmed since it has not been studied further nor were the experiments repeated (Bucala et al., 1985). Lyu et al. (2003) demonstrated that organ cultures of rat lenses treated with high dose GC for 7 days resulted in migration of LECs to the posterior region of the lens. However, treatment did not fully result in a posterior subcapsular cataract. Since GR knockout mice die shortly after birth, a conditional lens specific GR knockout mouse (using a CRE-recombinase and loxP system) would be needed to study GC effects (Cole et al., 2001; Matsumoto et al., 2003). However, it was suggested that the mouse and rat do not contain the GRβ, which could lead to different results in GR signaling compared to humans (Yudt and Cidlowski, 2001). If animal models are used to understand the mechanism of a lens GC response, verification of these results in human lenses is essential. Initial studies revealed that organ cultures of human lenses, cultured without serum to more closely resemble in vivo conditions, demonstrated similar changes identified in cells lines (Gupta et al., 2007). Furthermore, it would also be important to study these effects with both acute and chronic GC treatment, as well as in combination with other factors found in the lens environment to more closely resemble in vivo conditions.

The identification of a functional lens GR demonstrates that although GCs may have non-specific effects on the lens, a specific GC response must be understood in order to understand the pathogenesis, treatment and prevention of a steroid induced cataract. These novel insights provide the first clues into a GC mechanism of action. Further studies are necessary in order to understand the side effects of steroid therapy in lens epithelial cells.

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