REVIEW

Glucocorticoid receptor-mediated apoptosis: mechanisms of resistance in cancer cells

George Schlossmacher¹, Adam Stevens² and Anne White^{1,2}

¹Endocrinology and Diabetes, Faculty of Life Sciences and ²Faculty of Medical and Human Sciences, Manchester Academic Health Sciences Centre, University of Manchester, Manchester M13 9PT, UK

(Correspondence should be addressed to A White at Faculty of Life Sciences, University of Manchester; Email: anne.white@manchester.ac.uk)

Abstract

Glucocorticoids (Gcs) are commonly used to treat patients suffering from a wide range of cancers. Their main therapeutic role is based on Gc receptor (GR)-mediated mechanisms that trigger cell death but this varies depending on the cancer type. This review aims to provide an overview of the mechanisms of Gc-induced cell death and more importantly the changes in GR that lead to resistance to Gc treatment in cancer. The three main cancer types, which are susceptible to Gc resistance and therefore loss of Gc-induced apoptotic effects, are acute lymphoblastic leukaemia, osteosarcoma and small-cell lung carcinoma. A common theme is the loss of GR function and/or a downregulation of GR expression which leads to failure of the cell

death-inducing effects of Gcs. Loss of GR function is attributed to mutations in the GR gene, and in some cases a dominant-negative effect on any functional GR still present. The downregulation of GR expression can be due to decreased GR promoter activation, increased GR promoter methylation or increased expression of alternative splice isoforms of GR that have decreased transcriptional activity. Understanding the mechanisms behind Gc-triggered apoptosis and the resistance to it in these cancer types will help in further refining treatment regimens for patients and will decrease the chance of relapse caused by Gc-resistant cancer phenotypes.

Journal of Endocrinology (2011) **211**, 17–25

Glucocorticoid treatment of cancer

Glucocorticoids (Gcs) are widely used for the treatment of lymphoid malignancy (Pirrotte *et al.* 1997, Sionov *et al.* 2008) because of their dramatic effects on cell cycle progression and apoptosis. They are also used as a co-medication in the therapy of solid tumours, either because of their effectiveness in treating the malignancy, or for decreasing oedema, pain, electrolyte imbalance, nausea and emesis or to reduce cytotoxic reactions caused by other treatment regimens (Rutz 2002, Rutz & Herr 2004). The direct effects that Gcs have on cancer cells are varied but detailed studies have enabled a greater understanding of the effects they exert on cell death. Gcs are able to alter signalling in key survival pathways and this can result in reversible growth arrest or cell death in certain cell types.

Endogenous Gcs are involved in a variety of biological processes. In man, Gcs have important effects on metabolism, the immune response, development, the cardiovascular system and reproduction. However, one of the main functions of Gcs is that they reduce inflammatory responses. Because of

this, synthetic Gcs are used to treat symptoms of asthma, arthritis and dermatitis. At the cellular level, Gcs impact on processes ranging from proliferation and differentiation to apoptosis. It is this latter action that is important in their use as therapeutic agents in the treatment of cancer.

How do Gcs exert their effects at the cellular level?

Gcs are members of the steroid hormone family, which exert most of their effects by altering transcription of an array of steroid responsive genes. The transcriptional effects are mediated via the Gc receptor (GR), which is a member of the nuclear hormone receptor family.

Gcs bind GR, which then functions as a transcription factor to trigger genomic effects, however there is now evidence of faster effects, mediated non-genomically via membrane-associated receptors and their signalling cascades (Beck *et al.* 2009, Revollo & Cidlowski 2009). The GR gene generates several different splice and translation protein variants all of which are modular proteins that contain three distinct

functional regions: an N-terminal transactivation domain, a central DNA-binding domain and a C-terminal region that contains the hormone-binding site (Gross *et al.* 2009).

Genomic actions of Gcs are mediated by nuclear translocation of the GR (Fig. 1). In the absence of Gc ligand, cytoplasmic GR forms a heterocomplex with chaperone proteins which keeps the GR in an inactive state (Cadepond *et al.* 1991). Upon Gc binding, the GR dissociates from its chaperone proteins, homodimerises via the C-terminal ligand-binding domains (Bledsoe *et al.* 2002) and translocates to the nucleus. Once in the nucleus, the GR can then act as either a transcriptional activator or a repressor depending on the gene and the cellular environment.

GR dimers are able to bind to the Gc response elements (GREs) present in the promoter regions of the target genes. Once bound to the GRE, the GR recruits co-activator proteins that open the chromatin structure such as histone acetyltransferases. These include members of the p160 family of proteins (SRC1, PGC1, AIB1, etc.). Upregulation of transcription can usually be observed a few hours after the original Gc signal (Dean & Sanders 1996). GR can also upregulate gene transcription using mechanisms other than classical GREs; GR composite regulatory elements exist where GR interacts with DNA along with other transcription

factors such as c-Myb (Geng & Vedeckis 2005) and the GR can also bind to other DNA-bound transcription factors, a process termed 'tethering', and modulate their effects as seen with the Gc-mediated regulation of mitogen-activated protein kinases (MAPK)-phosphatase 1 (DUSP1) transcription where the GR effect is modulated by binding to promoter bound C/EBP β (Johansson-Haque *et al.* 2008).

Gc bound GR can also repress target genes via similar mechanisms to those used for activation of transcription, i.e. direct DNA binding via negative GREs (nGREs). This leads to recruitment of co-repressor proteins such as histone deacetylases, for example NCoR and SMRT, that 'close-up' chromatin to form a structure that does not favour transcription (Perissi *et al.* 2010). The GR is also able to repress transcription by DNA-based interactions with other transcription factors in composite elements, for example in Gc-mediated repression of the corticotropin-releasing hormone gene (Malkoski & Dorin 1999). Other mechanisms of repression include tethering other DNA-bound transcription factors such as in the repression of NF- κ B action (Nissen & Yamamoto 2000) and via competition with a transcriptional activator for DNA-binding sites such as repression of FasL expression by NF- κ B (Novac *et al.* 2006).

Figure 1 Glucocorticoid receptor signalling. The glucocorticoid receptor translocates to the nucleus upon ligand binding where it will act as a homo- or heterodimer with or without other co-factors to either repress or drive transcription of target genes.

Observations from transgenic animals that express a dimerisation-deficient mutation of the GR suggest that a proportion of the effects of Gcs are not modulated by dimerisation-dependent DNA interactions (Reichardt & Schütz 1998). Some of these effects can be explained by the modulation of cytoplasmic pathways via direct protein–protein interactions as shown for example with JNK (Löwenberg *et al.* 2008, Beck *et al.* 2009). Gcs exert a number of rapid actions that are independent of the regulation of gene transcription. Binding of Gcs to the GR stimulates phosphatidylinositol 3-kinase (PI3K) and the protein kinase AKT (Beck *et al.* 2009) in some cells. These effects result in rapid changes in the cytoplasmic environment that can lead to endothelial nitric oxide synthase (eNOS) activation and nitric oxide-dependent vasorelaxation (Hafezi-Moghadam *et al.* 2002) amongst other effects. Some of the immunosuppressive effects of Gcs are mediated by non-genomic signalling. In T cells, unliganded GR is associated in a complex with the antigen activated T cell receptor (TCR) and is necessary for TCR signalling. Gcs cause dissociation of the complex and result in TCR signalling blockade (Löwenberg *et al.* 2006).

GR is ubiquitously expressed although there are differences between tissues and the effect of Gcs in different tissues is influenced by a combination of the expression of different

splice variants and co-regulator proteins and the role of tissue-dependent post-translational modifications. This is exemplified by high expression levels of the GR-C3 isoform in bone cells that is associated with high levels of Gc-induced apoptosis (Lu *et al.* 2007).

Mechanisms of apoptosis relevant to Gcs

Programmed cell death, also referred to as apoptosis, is carried out by a family of proteins known as caspases (Fig. 2). Effector caspases are the final target in the signalling pathway referred to as the ‘caspase cascade’. When effector caspases become active, the cell will undergo apoptosis since their role includes mediating nuclear breakdown and cellular degradation. Effector caspases are activated by inducer caspases, which are tightly regulated by a complex network of signalling proteins.

There are two pathways which trigger apoptosis: the extrinsic pathway is dependent on ligand binding and subsequent activation of membrane-bound ‘death signal’ receptors. Activating these receptors triggers the caspase cascade, resulting in apoptosis (Elmore 2007). The second pathway is the more complex intrinsic pathway (Elmore 2007). This pathway is regulated by members of the Bcl-2

Figure 2 Mechanisms of glucocorticoid-mediated apoptosis. Glucocorticoids can exert their effects either genomically or non-genomically. While the exact pathway is not clear and may vary in different cell types it is thought that glucocorticoids act via the mitochondrial pathway to cause caspase activation.

family consisting of pro- and anti-apoptotic proteins. Members of the Bcl-2 family control many forms of apoptotic cell death by 'tipping the balance' from anti- to pro-apoptotic Bcl-2 family members. This balance is termed the 'Bcl-2 rheostat' (Ploner *et al.* 2008). Cellular stress, such as DNA damage, leads to an increase in pro-apoptotic protein levels which in turn allows pro-apoptotic Bcl-2 proteins to induce the release of cytochrome *c* from mitochondria, causing activation of the caspase cascade (Adams & Cory 1998).

Effects of Gcs on apoptosis

The mechanism behind Gc-induced apoptosis is not fully understood and seems to vary depending on cell type. The levels of GR expression are important as evidenced by work in transgenic mice with increased (Reichardt *et al.* 2000) and decreased GR expression (Pazirandeh *et al.* 2002). Gcs can prevent activated upregulation of FasL but not Fas signalling as such, demonstrating that GR interferes with FasL expression in T cell hybridomas (D'Adamio *et al.* 1997, Ashwell *et al.* 2000). This indicates that Gcs can act on the extrinsic pathway.

There is considerable evidence that in almost all cell types studied, Gcs act via the intrinsic pathway. Gcs can activate cell death through induction of pro-apoptotic members of the Bcl-2 family, such as Bim, Bid and Bad (Han *et al.* 2001, Wang *et al.* 2003, Lu *et al.* 2007) and/or repression of anti-apoptotic members, such as Bcl-2, Mcl-1 and Bcl-xL, (Rogatsky *et al.* 1999, Casale *et al.* 2003, Chauhan *et al.* 2003, Lu *et al.* 2007). Gc-induced apoptosis is also compromised in thymocytes from mice deficient in pro-apoptotic proteins Apaf1 (Yoshida *et al.* 1998) or caspase 9 (Kuidera *et al.* 1998). In addition, thymocytes from double knockout mice lacking *Bak* and *Bax* are resistant to Gc-induced apoptosis (Rathmell *et al.* 2002).

In addition to the well-described nuclear mechanisms of Gc action, steroid hormones have also been shown to directly or indirectly affect mitochondrial function (Manoli *et al.* 2007). In several cell lines, GR translocation to the mitochondria was observed in correlation with susceptibility to Gc-induced apoptosis via the ligand-induced mitochondrial pathway rather than nuclear translocation of the GR (Sionov *et al.* 2006a,b). However, mitochondrial translocation of GR was only observed in cells responsive to Gc-induced apoptosis, and not in Gc-resistant types. This presents a qualitative difference in GR behaviour that may explain the differential response of some cancer cells to Gc therapy.

Gc-induced apoptosis in tumour cells and mechanisms of resistance

Most of the data obtained so far on the mechanisms of Gc-induced apoptosis in tumour cells is from work done in acute lymphoblastic leukaemia (ALL), osteosarcoma and small-cell lung cancer. In all of these tumour types there are examples of resistance to Gc actions. In fact, most cancer cells

derived from malignant solid tumours seem to exhibit resistance towards Gc-induced apoptosis, which makes the comparison between different tumour types even more important (Herr *et al.* 2009).

Resistance to Gc-induced apoptosis in lymphoblastic leukaemia

There are extensive studies outlining the mechanisms by which Gcs cause cell death in ALL. It is well known that Gcs induce apoptosis in some lymphoid cells, such as immature thymocytes and ALL cells (Smith & Cidlowski 2010) and this has subsequently led to treating lymphoid malignancies with Gcs (Gaynon & Carrel 1999). However, resistance to Gcs has been identified as a significant feature during treatment and insight is needed on how resistance develops in these tumour cells to improve Gc-mediated therapies in haematological malignancies.

The mechanisms by which Gcs induce apoptosis in ALL are not clearly defined and this makes understanding of acquired resistance difficult. It is known that Gc-mediated apoptosis in ALL requires the interaction of the Gc ligand with its receptor. This is exemplified by failure of apoptotic induction in human ALL cell lines expressing mutant forms of GR (Hala *et al.* 1996). However, Gc sensitivity in resistant ALL cell lines can be restored by introducing exogenous wild-type GR (Helmberg *et al.* 1995, Geley *et al.* 1996). Further studies in human T-ALL cell lines expressing differing levels of GR showed that GR expression above a certain threshold is vital for Gc sensitivity (Geley *et al.* 1996) and similar observations were later made in Gc-sensitive and Gc-resistant multiple myeloma lines (Chauhan *et al.* 2002). Interestingly, washing out Gcs before 24 h prevented ALL cells from undergoing apoptosis (Brunet *et al.* 1998). This suggests that GR levels above a certain threshold and appropriate ligand availability are required for some time to cause apoptosis triggering in ALL cells.

Gc-triggered apoptosis is believed to occur via changes in gene expression but discerning if this is due to transactivation, transrepression or a combination of the two proves to be difficult. Gc-resistant Jurkat cells and CEM cells were sensitised to Gcs by expressing transactivation-deficient GR mutants constitutively, leading to the conclusion that transrepression alone was sufficient for apoptosis (Thompson *et al.* 1992, Helmberg *et al.* 1995). However, the opposite result was presented from work done in mouse thymoma cells deficient for GR, which were transfected with N-terminal-deleted GR constructs (Dieken & Miesfeld 1992). In this instance transactivation was at least partially required as cells were only able to undergo apoptosis once a VP-16 transactivation domain had been transfected in. VP-16 is thus acting as a substitute for the missing GR transactivation domain. Based on these contradictory findings it appears that upon Gc binding to GR there are multiple mechanisms of altering gene expression in favour of pro-apoptotic signals.

The likelihood that instead of a single pathway causing Gc-induced apoptosis in ALL there are multiple mechanisms, depending on cell origin and tissue environment, poses a problem when it comes to pinpointing target genes involved in triggering Gc-mediated apoptosis. Gcs may activate apoptosis through direct regulation of components of either the extrinsic or intrinsic apoptotic pathway. Inhibition of caspase 8 in human ALL cell lines did not prevent the cells from undergoing apoptosis (Geley *et al.* 1997, Planey *et al.* 2003) and this may indicate that the extrinsic pathway is not necessary for Gc-mediated apoptosis. Studies in human ALL cell lines showed that members of the anti-apoptotic Bcl-2 protein family were able to diminish Gc-mediated apoptosis in human ALL cell lines when overexpressed (Brunet *et al.* 1998, Hartmann *et al.* 1999). It has become evident that Gc-mediated apoptosis includes the upregulation of pro-apoptotic (Han *et al.* 2001, Wang *et al.* 2003) and/or downregulation of anti-apoptotic genes (Chauhan *et al.* 2002, Casale *et al.* 2003) and this would therefore suggest that altering the expression levels of proteins involved in the Bcl-2 rheostat provides a mechanism for explaining Gc-mediated apoptosis (Schmidt *et al.* 2004).

In some cases, it is possible that the GR can cause apoptosis through non-genomic mechanisms, for example by the rapid activation of protein kinases such as MAPK, PI3K and AKT, which in turn activates eNOS (Limbourg & Liao 2003).

Mechanisms of Gc resistance in lymphoid malignancies

The necessity for prolonged treatment with Gcs in ALL makes it prone to development of Gc resistance. In addition, those patients who relapse have increased Gc resistance (Schrappe *et al.* 2000) and this resistance is associated with poor prognosis. In various malignant cell lines resistance is caused by mutations in the GR (Ashraf & Thompson 1993, Powers *et al.* 1993, Strasser-Wozak *et al.* 1995, Hala *et al.* 1996, Riml *et al.* 2004, Schmidt *et al.* 2006). However, there are only a few examples of GR mutations causing a resistant phenotype in primary cells from cancer patients displaying Gc resistance. To date, the only known acquired GR mutations associated with Gc resistance in *in vivo* malignancies are the L753F and Δ 702 mutants in the ligand-binding domain of the GR. L753F was discovered in cells from a patient with Gc-resistant ALL with the mutant being transactivation deficient (Hillmann *et al.* 2000). The Δ 702 mutant was also discovered in cells from a patient with Gc-resistant ALL and this mutant has a decreased affinity for ligand binding (Irving *et al.* 2005).

Resistance to Gcs can occur by downregulation of the GR following Gc treatment (Kfir *et al.* 2007). Decreased expression of the GR may occur through decreased activity of the GR promoter, decreased mRNA stability (Schaaf & Cidlowski 2002), or decreased protein stability (Wallace & Cidlowski 2001). Furthermore, there is evidence of

alterations in the expression levels of GR isoforms, which might contribute towards Gc resistance. GR β expression is increased in CEM cells and Gc-resistant CLL (Haarman *et al.* 2004), although there was no correlation between Gc resistance and GR β expression in primary cells from patients with ALL (Haarman *et al.* 2004) where there was a correlation with GR γ expression. There is also increased GR-P expression in Gc-resistant tumour cells but not primary ALL cells (Tissing *et al.* 2005).

Given the plethora of potential changes in the GR in resistant ALL cells it may be necessary to focus on targets further downstream, such as modulation of Bim expression, to increase apoptosis. However, yet again these pathways seem very diverse and whether a single mechanism will overcome the resistance remains controversial (Smith & Cidlowski 2010).

Resistance to Gc-induced apoptosis in osteosarcoma cells

It has been suggested that Gc resistance is a hallmark of osteosarcoma development due to the fact that Gcs cause apoptosis in osteoblast cells (Weinstein *et al.* 1998). In an animal model where 11- β -hydroxysteroid dehydrogenase 2, an enzyme that reduces corticosteroid levels, is overexpressed, it appears that Gcs exert their apoptotic effect directly on bone cells (O'Brien *et al.* 2004). The drive to remove GR from the cell in the human osteosarcoma cell line, U2OS, negates any Gc-induced effects on inhibition of proliferation via G1 cycle arrest (Rogatsky *et al.* 1997, 2003, Takayama *et al.* 2006). The pathway of the apoptosis trigger in U2OS cells is however still largely unknown. There is evidence that decreased Bcl-2 may be responsible for Gc-mediated apoptosis (Rogatsky *et al.* 1999) and downregulation of anti-apoptotic factors such as Bcl-xL and Mcl-1 has been observed in U2OS cells undergoing apoptosis (Lu *et al.* 2007). Another complexity in Gc-mediated apoptosis in osteosarcoma cells occurs due to specific effects of different GR isoforms produced via alternative translation initiation mechanisms, which result in varying lengths of the N-terminus of GR (Lu & Cidlowski 2005). The GR-C isoform was more active in regulating genes involved in apoptosis that may be due to selective recruitment of co-factors (Lu *et al.* 2007), while expression of the GR-D isoform reduced Gc-mediated cell death suggesting that expression of this isoform may lead to Gc resistance.

Resistance to Gc-induced apoptosis in small-cell lung cancer cells

Some patients with small-cell lung cancer (SCLC) develop the ectopic ACTH syndrome because their tumours secrete the hormone ACTH (or in most cases, its prohormone, proopiomelanocortin (POMC)). In the pituitary, expression of the POMC prohormone and ACTH secretion is inhibited by Gcs in a negative feedback loop. However, SCLC tumours

are resistant to Gc inhibition and this has long been used as the basis of one of the main diagnostic tests for this syndrome (Oliver *et al.* 2003).

This led to the study of Gc resistance in a panel of SCLC cell lines that secrete POMC (Stewart *et al.* 1989, White *et al.* 1989, Clark *et al.* 1990). A common theme that became apparent was that the functionality of the GR was impaired. This occurred in one cell line because of a mutation in the GR gene resulting in the truncated GR form known as GR δ (also known as GR-P) being expressed (Gaitan *et al.* 1995, Turney & Kovacs 2001). In another cell line, one allele of the GR had an amino acid transition in the zinc finger domain of the DNA-binding region of the GR, which resulted in GR γ (Ray *et al.* 1994, 1996). In addition, the overexpression of the GR co-repressor NCoR leads to reduced transactivation and is therefore believed to be responsible for Gc resistance in the SCLC cell line, CORL 103 (Waters *et al.* 2004). This is in line with other observations that point towards abnormal co-regulator expression as a mechanism in cancers and metabolic disease including Gc resistance (Lonard *et al.* 2007).

Recently, we have identified an overall decrease in GR expression across a panel of SCLC cell lines (Sommer *et al.* 2007 and unpublished data).

As the SCLC cell lines possess reduced GR functionality, the effects of restoring expression were investigated. Transient transfection of wild-type GR restores Gc sensitivity in the SCLC cell line, DMS 79 (Ray *et al.* 1994, Sommer *et al.* 2007). Treatment with Gcs had an inhibitory effect on proliferation in a non-SCLC cell line, but not in DMS 79 cells. When an exogenous form of the GR was overexpressed in DMS 79 cells via retroviral infection, a significant increase in cell death was observed, which was inhibited with the GR antagonist RU486. Transient overexpression of GR in DMS 79 cells resulted in >90% loss in live cells within 72 h. Microarray analysis demonstrated that GR overexpression caused an increase in expression levels of the pro-apoptotic genes, *Bad* and *Bax* (Sommer *et al.* 2007).

This pro-apoptotic effect of GR was substantiated *in vivo* in SCLC xenografts that were infected with a GR-expressing adenovirus that caused significantly decreased tumour growth (Sommer *et al.* 2010). A significant increase in TUNEL-positive cells was observed. Interestingly, a number of uninfected cells were also shown to be apoptotic, suggesting a bystander effect on surrounding cells. The GR-induced apoptosis in DMS 79 xenografts appeared to involve decreased expression of the anti-apoptotic genes Bcl-2, Bcl-xL and Mcl-1 (Sommer *et al.* 2010).

Low level expression of GR in the panel of SCLC cell lines, which is associated with increased promoter methylation (unpublished data), is similar to mechanisms associated with conventional tumour suppressor genes. Understanding how restoration of GR expression results in tumour cell death may uncover new therapeutic strategies in SCLC treatment in the future.

Summary

The pleomorphic effects of Gcs are underpinned by a wide range of mechanisms that mediate Gc actions in different cell types. Not surprisingly, this has led to multiple mechanisms being implicated in Gc resistance making it difficult to identify ways to overcome Gc resistance in patients. This is particularly important as Gcs are one of the most effective agents for lymphoid malignancies and are used to treat many cancers not only as pro-apoptotic agents but also to reduce emesis associated with chemotherapy. There is a possibility that treatment with Gcs induces the resistant phenotype in cells of solid tumours while having pro-apoptotic properties in lymphoid malignancies. This would imply that Gcs may be crucial in causing faster growth and metastasis of solid tumours by providing a selection pressure. However, since there are examples of Gc resistance in lymphoid tumour cell lines it may be that solid tumours become resistant more easily due to their growth characteristics, whereas lymphoid tumours take longer to develop resistance because of their morphology. If we are to find therapeutic approaches to combat Gc resistance we will need a greater understanding of key checkpoints in these Gc-regulated pathways.

Declaration of interest

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

Funding

G S is funded by the BBSRC and a Barbara Mawer Trust studentship.

References

- Adams JM & Cory S 1998 The Bcl-2 protein family: arbiters of cell survival. *Science* **281** 1322–1326. (doi:10.1126/science.281.5381.1322)
- Ashraf J & Thompson EB 1993 Identification of the activation-labile gene: a single point mutation in the human glucocorticoid receptor presents as two distinct receptor phenotypes. *Molecular Endocrinology* **7** 631–642. (doi:10.1210/me.7.5.631)
- Ashwell JD, Lu FW & Vacchio MS 2000 Glucocorticoids in T cell development and function*. *Annual Review of Immunology* **18** 309–345. (doi:10.1146/annurev.immunol.18.1.309)
- Beck IM, Vanden Berghe W, Vermeulen L, Yamamoto KR, Haegeman G & De Bosscher K 2009 Crosstalk in inflammation: the interplay of glucocorticoid receptor-based mechanisms and kinases and phosphatases. *Endocrine Reviews* **30** 830–882. (doi:10.1210/er.2009-0013)
- Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, McKee DD, Consler TG, Parks DJ, Stewart EL, Willson TM *et al.* 2002 Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell* **110** 93–105. (doi:10.1016/S0092-8674(02)00817-6)
- Brunet CL, Gunby RH, Benson RS, Hickman JA, Watson AJ & Brady G 1998 Commitment to cell death measured by loss of clonogenicity is separable from the appearance of apoptotic markers. *Cell Death and Differentiation* **5** 107–115. (doi:10.1038/sj.cdd.4400334)

- Cadepond F, Schweizer-Groyer G, Segard-Maurel I, Jibard N, Hollenberg SM, Giguère V, Evans RM & Baulieu EE 1991 Heat shock protein 90 as a critical factor in maintaining glucocorticosteroid receptor in a nonfunctional state. *Journal of Biological Chemistry* **266** 5834–5841.
- Casale F, Addeo R, D'Angelo V, Indolfi P, Poggi V, Morgera C, Crisci S & Di Tullio MT 2003 Determination of the *in vivo* effects of prednisone on Bcl-2 family protein expression in childhood acute lymphoblastic leukemia. *International Journal of Oncology* **22** 123–128.
- Chauhan D, Catley L, Hideshima T, Li G, Leblanc R, Gupta D, Sattler M, Richardson P, Schlossman RL, Podar K *et al.* 2002 2-Methoxyestradiol overcomes drug resistance in multiple myeloma cells. *Blood* **100** 2187–2194. (doi:10.1182/blood-2002-02-0376)
- Chauhan S, Leach CH, Kunz S, Bloom JW & Miesfeld RL 2003 Glucocorticoid regulation of human eosinophil gene expression. *Journal of Steroid Biochemistry and Molecular Biology* **84** 441–452. (doi:10.1016/S0960-0760(03)00065-7)
- Clark AJ, Stewart ME, Lavender PM, Farrell W, Crosby SR, Rees LH & White A 1990 Defective glucocorticoid regulation of proopiomelanocortin gene expression and peptide secretion in a small cell lung cancer cell line. *Journal of Clinical Endocrinology and Metabolism* **70** 485–490. (doi:10.1210/jcem-70-2-485)
- D'Adamo F, Zollo O, Moraca R, Ayroldi E, Bruscoli S, Bartoli A, Cannarile L, Migliorati G & Riccardi C 1997 A new dexamethasone-induced gene of the leucine zipper family protects T lymphocytes from TCR/CD3-activated cell death. *Immunity* **7** 803–812. (doi:10.1016/S1074-7613(00)80398-2)
- Dean DM & Sanders MM 1996 Ten years after: reclassification of steroid-responsive genes. *Molecular Endocrinology* **10** 1489–1495. (doi:10.1210/me.10.12.1489)
- Dieken ES & Miesfeld RL 1992 Transcriptional transactivation functions localized to the glucocorticoid receptor N terminus are necessary for steroid induction of lymphocyte apoptosis. *Molecular and Cellular Biology* **12** 589–597.
- Elmore S 2007 Apoptosis: a review of programmed cell death. *Toxicologic Pathology* **35** 495–516. (doi:10.1080/0196230701320337)
- Gaitan D, DeBold CR, Turney MK, Zhou P, Orth DN & Kovacs WJ 1995 Glucocorticoid receptor structure and function in an adrenocorticotropin-secreting small cell lung cancer. *Molecular Endocrinology* **9** 1193–1201. (doi:10.1210/me.9.9.1193)
- Gaynon PS & Carrel AL 1999 Glucocorticosteroid therapy in childhood acute lymphoblastic leukemia. *Advances in Experimental Medicine and Biology* **457** 593–605.
- Geley S, Hartmann BL, Hala M, Strasser-Wozak EM, Kapelari K & Kofler R 1996 Resistance to glucocorticoid-induced apoptosis in human T-cell acute lymphoblastic leukemia CEM-C1 cells is due to insufficient glucocorticoid receptor expression. *Cancer Research* **56** 5033–5038.
- Geley S, Hartmann BL, Kapelari K, Egle A, Villunger A, Heidacher D, Greil R, Auer B & Kofler R 1997 The interleukin 1 β -converting enzyme inhibitor CrmA prevents Apo1/Fas- but not glucocorticoid-induced poly(ADP-ribose) polymerase cleavage and apoptosis in lymphoblastic leukemia cells. *FEBS Letters* **402** 36–40. (doi:10.1016/S0014-5793(96)01496-2)
- Geng CD & Vedeckis WV 2005 c-Myb and members of the c-Ets family of transcription factors act as molecular switches to mediate opposite steroid regulation of the human glucocorticoid receptor 1A promoter. *Journal of Biological Chemistry* **280** 43264–43271. (doi:10.1074/jbc.M508245200)
- Gross KL, Lu NZ & Cidlowski JA 2009 Molecular mechanisms regulating glucocorticoid sensitivity and resistance. *Molecular and Cellular Endocrinology* **300** 7–16. (doi:10.1016/j.mce.2008.10.001)
- Haarman EG, Kaspers GJ, Pieters R, Rottier MM & Veerman AJ 2004 Glucocorticoid receptor alpha, beta and gamma expression vs *in vitro* glucocorticoid resistance in childhood leukemia. *Leukemia* **18** 530–537. (doi:10.1038/sj.leu.2403225)
- Hafezi-Moghadam A, Simoncini T, Yang Z, Limbourg FP, Plumier JC, Rebsamen MC, Hsieh CM, Chui DS, Thomas KL, Prorock AJ *et al.* 2002 Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. *Nature Medicine* **8** 473–479. (doi:10.1038/nm0502-473)
- Hala M, Hartmann BL, Böck G, Geley S & Kofler R 1996 Glucocorticoid-receptor-gene defects and resistance to glucocorticoid-induced apoptosis in human leukemic cell lines. *International Journal of Cancer* **68** 663–668. (doi:10.1002/(SICI)1097-0215(19961127)68:5<663::AID-IJC17>3.0.CO;2-2)
- Han J, Flemington C, Houghton AB, Gu Z, Zambetti GP, Lutz RJ, Zhu L & Chittenden T 2001 Expression of bbc3, a pro-apoptotic BH3-only gene, is regulated by diverse cell death and survival signals. *PNAS* **98** 11318–11323. (doi:10.1073/pnas.201208798)
- Hartmann BL, Geley S, Löffler M, Hattmannstorfer R, Strasser-Wozak EM, Auer B & Kofler R 1999 Bcl-2 interferes with the execution phase, but not upstream events, in glucocorticoid-induced leukemia apoptosis. *Oncogene* **18** 713–719. (doi:10.1038/sj.onc.1202339)
- Helmberg A, Auphan N, Caelles C & Karin M 1995 Glucocorticoid-induced apoptosis of human leukemic cells is caused by the repressive function of the glucocorticoid receptor. *EMBO Journal* **14** 452–460.
- Herr I, Büchler MW & Mattern J 2009 Glucocorticoid-mediated apoptosis resistance of solid tumors. *Results and Problems in Cell Differentiation* **49** 191–218. (doi:10.1007/400_2008_20)
- Hillmann AG, Ramdas J, Multanen K, Norman MR & Harmon JM 2000 Glucocorticoid receptor gene mutations in leukemic cells acquired *in vitro* and *in vivo*. *Cancer Research* **60** 2056–2062.
- Irving JA, Minto L, Bailey S & Hall AG 2005 Loss of heterozygosity and somatic mutations of the glucocorticoid receptor gene are rarely found at relapse in pediatric acute lymphoblastic leukemia but may occur in a subpopulation early in the disease course. *Cancer Research* **65** 9712–9718. (doi:10.1158/0008-5472.CAN-05-1227)
- Johansson-Haque K, Palanichamy E & Okret S 2008 Stimulation of MAPK-phosphatase 1 gene expression by glucocorticoids occurs through a tethering mechanism involving C/EBP. *Journal of Molecular Endocrinology* **41** 239–249. (doi:10.1677/JME-08-0015)
- Kfir S, Sionov RV, Zafir E, Zilberman Y & Yefenof E 2007 Staurosporine sensitizes T lymphoma cells to glucocorticoid-induced apoptosis: role of Nur77 and Bcl-2. *Cell Cycle* **6** 3086–3096. (doi:10.4161/cc.6.24.5023)
- Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P & Flavell RA 1998 Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell* **94** 325–337. (doi:10.1016/S0092-8674(00)81476-2)
- Limbourg FP & Liao JK 2003 Nontranscriptional actions of the glucocorticoid receptor. *Journal of Molecular Medicine* **81** 168–174. (doi:10.1007/s00109-003-0418-y)
- Lonard DM, Lanz RB & O'Malley BW 2007 Nuclear receptor coregulators and human disease. *Endocrine Reviews* **28** 575–587. (doi:10.1210/er.2007-0012)
- Löwenberg M, Verhaar AP, Bilderbeek J, Marle J, Buttgerit F, Peppelenbosch MP, van Deventer SJ & Hommes DW 2006 Glucocorticoids cause rapid dissociation of a T-cell-receptor-associated protein complex containing LCK and FYN. *EMBO Reports* **7** 1023–1029. (doi:10.1038/sj.embor.7400775)
- Löwenberg M, Stahn C, Hommes DW & Buttgerit F 2008 Novel insights into mechanisms of glucocorticoid action and the development of new glucocorticoid receptor ligands. *Steroids* **73** 1025–1029. (doi:10.1016/j.steroids.2007.12.002)
- Lu NZ & Cidlowski JA 2005 Translational regulatory mechanisms generate N-terminal glucocorticoid receptor isoforms with unique transcriptional target genes. *Molecular Cell* **18** 331–342. (doi:10.1016/j.molcel.2005.03.025)
- Lu NZ, Collins JB, Grissom SF & Cidlowski JA 2007 Selective regulation of bone cell apoptosis by translational isoforms of the glucocorticoid receptor. *Molecular and Cellular Biology* **27** 7143–7160. (doi:10.1128/MCB.00253-07)
- Malkoski SP & Dorin RI 1999 Composite glucocorticoid regulation at a functionally defined negative glucocorticoid response element of the human corticotropin-releasing hormone gene. *Molecular Endocrinology* **13** 1629–1644. (doi:10.1210/me.13.10.1629)
- Manoli I, Alesci S, Blackman MR, Su YA, Rennert OM & Chrousos GP 2007 Mitochondria as key components of the stress response. *Trends in Endocrinology and Metabolism* **18** 190–198. (doi:10.1016/j.tem.2007.04.004)

- Nissen RM & Yamamoto KR 2000 The glucocorticoid receptor inhibits NFκB by interfering with serine-2 phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes and Development* **14** 2314–2329. (doi:10.1101/gad.827900)
- Novac N, Baus D, Dostert A & Heinzel T 2006 Competition between glucocorticoid receptor and NFκB for control of the human FasL promoter. *FASEB Journal* **20** 1074–1081. (doi:10.1096/fj.05-5457com)
- O'Brien CA, Jia D, Plotkin LI, Bellido T, Powers CC, Stewart SA, Manolagas SC & Weinstein RS 2004 Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. *Endocrinology* **145** 1835–1841. (doi:10.1210/en.2003-0990)
- Oliver RL, Davis JR & White A 2003 Characterisation of ACTH related peptides in ectopic Cushing's syndrome. *Pituitary* **6** 119–126. (doi:10.1023/B:PITU.0000011172.26649.df)
- Pazirandeh A, Xue Y, Prestegard T, Jondal M & Okret S 2002 Effects of altered glucocorticoid sensitivity in the T cell lineage on thymocyte and T cell homeostasis. *FASEB Journal* **16** 727–729. (doi:10.1096/fj.01-0891fje)
- Perissi V, Jepsen K, Glass CK & Rosenfeld MG 2010 Deconstructing repression: evolving models of co-repressor action. *Nature Reviews. Genetics* **11** 109–123. (doi:10.1038/nrg2736)
- Pirrotte B, Levivier M, Goldman S, Brucher JM, Brothi J & Hildebrand J 1997 Glucocorticoid-induced long-term remission in primary cerebral lymphoma: case report and review of the literature. *Journal of Neuro-Oncology* **32** 63–69. (doi:10.1023/A:1005733416571)
- Planey SL, Abrams MT, Robertson NM & Litwack G 2003 Role of apical caspases and glucocorticoid-regulated genes in glucocorticoid-induced apoptosis of pre-B leukemic cells. *Cancer Research* **63** 172–178.
- Ploner C, Rainer J, Niederregger H, Eduardoff M, Villunger A, Geley S & Kofler R 2008 The BCL2 rheostat in glucocorticoid-induced apoptosis of acute lymphoblastic leukemia. *Leukemia* **22** 370–377. (doi:10.1038/sj.leu.2405039)
- Powers JH, Hillmann AG, Tang DC & Harmon JM 1993 Cloning and expression of mutant glucocorticoid receptors from glucocorticoid-sensitive and -resistant human leukemic cells. *Cancer Research* **53** 4059–4065.
- Rathmell JC, Lindsten T, Zong WX, Cinalli RM & Thompson CB 2002 Deficiency in Bak and Bax perturbs thymic selection and lymphoid homeostasis. *Nature Immunology* **3** 932–939. (doi:10.1038/ni834)
- Ray DW, Littlewood AC, Clark AJ, Davis JR & White A 1994 Human small cell lung cancer cell lines expressing the proopiomelanocortin gene have aberrant glucocorticoid receptor function. *Journal of Clinical Investigation* **93** 1625–1630. (doi:10.1172/JCI117143)
- Ray DW, Davis JR, White A & Clark AJ 1996 Glucocorticoid receptor structure and function in glucocorticoid-resistant small cell lung carcinoma cells. *Cancer Research* **56** 3276–3280.
- Reichardt HM & Schütz G 1998 Glucocorticoid signalling – multiple variations of a common theme. *Molecular and Cellular Endocrinology* **146** 1–6. (doi:10.1016/S0303-7207(98)00208-1)
- Reichardt HM, Umland T, Bauer A, Kretz O & Schütz G 2000 Mice with an increased glucocorticoid receptor gene dosage show enhanced resistance to stress and endotoxic shock. *Molecular and Cellular Biology* **20** 9009–9017. (doi:10.1128/MCB.20.23.9009-9017.2000)
- Revollo JR & Cidlowski JA 2009 Mechanisms generating diversity in glucocorticoid receptor signaling. *Annals of the New York Academy of Sciences* **1179** 167–178. (doi:10.1111/j.1749-6632.2009.04986.x)
- Riml S, Schmidt S, Ausserlechner MJ, Geley S & Kofler R 2004 Glucocorticoid receptor heterozygosity combined with lack of receptor auto-induction causes glucocorticoid resistance in Jurkat acute lymphoblastic leukemia cells. *Cell Death and Differentiation* **11** (Supplement 1) S65–S72. (doi:10.1038/sj.cdd.4401413)
- Rogatsky I, Trowbridge JM & Garabedian MJ 1997 Glucocorticoid receptor-mediated cell cycle arrest is achieved through distinct cell-specific transcriptional regulatory mechanisms. *Molecular and Cellular Biology* **17** 3181–3193.
- Rogatsky I, Hittelman AB, Pearce D & Garabedian MJ 1999 Distinct glucocorticoid receptor transcriptional regulatory surfaces mediate the cytotoxic and cytostatic effects of glucocorticoids. *Molecular and Cellular Biology* **19** 5036–5049.
- Rogatsky I, Wang JC, Derynck MK, Nonaka DF, Khodabakhsh DB, Haqq CM, Darimont BD, Garabedian MJ & Yamamoto KR 2003 Target-specific utilization of transcriptional regulatory surfaces by the glucocorticoid receptor. *PNAS* **100** 13845–13850. (doi:10.1073/pnas.2336092100)
- Rutz HP 2002 Effects of corticosteroid use on treatment of solid tumours. *Lancet* **360** 1969–1970. (doi:10.1016/S0140-6736(02)11922-2)
- Rutz HP & Herr I 2004 Interference of glucocorticoids with apoptosis signaling and host-tumor interactions. *Cancer Biology and Therapy* **3** 715–718. (doi:10.4161/cbt.3.8.966)
- Schaaf MJ & Cidlowski JA 2002 AUUUA motifs in the 3'UTR of human glucocorticoid receptor alpha and beta mRNA destabilize mRNA and decrease receptor protein expression. *Steroids* **67** 627–636. (doi:10.1016/S0039-128X(02)00015-6)
- Schmidt S, Rainer J, Ploner C, Presul E, Riml S & Kofler R 2004 Glucocorticoid-induced apoptosis and glucocorticoid resistance: molecular mechanisms and clinical relevance. *Cell Death and Differentiation* **11** S45–S55. (doi:10.1038/sj.cdd.4401456)
- Schmidt S, Irving JA, Minto L, Matheson E, Nicholson L, Ploner A, Parson W, Kofler A, Amort M, Erdel M *et al.* 2006 Glucocorticoid resistance in two key models of acute lymphoblastic leukemia occurs at the level of the glucocorticoid receptor. *FASEB Journal* **20** 2600–2602. (doi:10.1096/fj.06-6214fje)
- Schrappé M, Reiter A, Zimmermann M, Harbott J, Ludwig WD, Henze G, Gadner H, Odenwald E & Riehm H 2000 Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. Berlin-Frankfurt-Münster. *Leukemia* **14** 2205–2222. (doi:10.1038/sj.leu.2401973)
- Sionov RV, Cohen O, Kfir S, Zilberman Y & Yefenof E 2006a Role of mitochondrial glucocorticoid receptor in glucocorticoid-induced apoptosis. *Journal of Experimental Medicine* **203** 189–201. (doi:10.1084/jem.20050433)
- Sionov RV, Kfir S, Zafir E, Cohen O, Zilberman Y & Yefenof E 2006b Glucocorticoid-induced apoptosis revisited: a novel role for glucocorticoid receptor translocation to the mitochondria. *Cell Cycle* **5** 1017–1026. (doi:10.4161/cc.5.10.2738)
- Sionov RV, Spokoini R, Kfir-Erenfeld S, Cohen O & Yefenof E 2008 Mechanisms regulating the susceptibility of hematopoietic malignancies to glucocorticoid-induced apoptosis. *Advances in Cancer Research* **101** 127–248. (doi:10.1016/S0065-230X(08)00406-5)
- Smith LK & Cidlowski JA 2010 Glucocorticoid-induced apoptosis of healthy and malignant lymphocytes. *Progress in Brain Research* **182** 1–30. (doi:10.1016/S0079-6123(10)82001-1)
- Sommer P, Le Rouzic P, Gillingham H, Berry A, Kayahara M, Huynh T, White A & Ray DW 2007 Glucocorticoid receptor overexpression exerts an antisurvival effect on human small cell lung cancer cells. *Oncogene* **26** 7111–7121. (doi:10.1038/sj.onc.1210524)
- Sommer P, Cowen RL, Berry A, Cookson A, Telfer BA, Williams KJ, Stratford IJ, Kay P, White A & Ray DW 2010 Glucocorticoid receptor over-expression promotes human small cell lung cancer apoptosis *in vivo* and thereby slows tumor growth. *Endocrine-Related Cancer* **17** 203–213. (doi:10.1677/ERC-09-0241)
- Stewart MF, Crosby SR, Gibson S, Twentyman PR & White A 1989 Small cell lung cancer cell lines secrete predominantly ACTH precursor peptides not ACTH. *British Journal of Cancer* **60** 20–24. (doi:10.1038/bjc.1989.212)
- Strasser-Wozak EM, Hattmannstorfer R, Hala M, Hartmann BL, Fiegl M, Geley S & Kofler R 1995 Splice site mutation in the glucocorticoid receptor gene causes resistance to glucocorticoid-induced apoptosis in a human acute leukemic cell line. *Cancer Research* **55** 348–353.
- Takayama S, Rogatsky I, Schwarcz LE & Darimont BD 2006 The glucocorticoid receptor represses cyclin D1 by targeting the Tcf-beta-catenin complex. *Journal of Biological Chemistry* **281** 17856–17863. (doi:10.1074/jbc.M602290200)
- Thompson EB, Nazareth LV, Thulasi R, Ashraf J, Harbour D & Johnson BH 1992 Glucocorticoids in malignant lymphoid cells: gene regulation and the minimum receptor fragment for lysis. *Journal of Steroid Biochemistry and Molecular Biology* **41** 273–282. (doi:10.1016/0960-0760(92)90352-J)

- Tissing WJ, Lauten M, Meijerink JP, den Boer ML, Koper JW, Sonneveld P & Pieters R 2005 Expression of the glucocorticoid receptor and its isoforms in relation to glucocorticoid resistance in childhood acute lymphocytic leukemia. *Haematologica* **90** 1279–1281.
- Turney MK & Kovacs WJ 2001 Function of a truncated glucocorticoid receptor form at a negative glucocorticoid response element in the proopiomelanocortin gene. *Journal of Molecular Endocrinology* **26** 43–49. (doi:10.1677/jme.0.0260043)
- Wallace AD & Cidlowski JA 2001 Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. *Journal of Biological Chemistry* **276** 42714–42721. (doi:10.1074/jbc.M106033200)
- Wang Z, Malone MH, He H, McColl KS & Distelhorst CW 2003 Microarray analysis uncovers the induction of the proapoptotic BH3-only protein Bim in multiple models of glucocorticoid-induced apoptosis. *Journal of Biological Chemistry* **278** 23861–23867. (doi:10.1074/jbc.M301843200)
- Waters CE, Stevens A, White A & Ray DW 2004 Analysis of co-factor function in a glucocorticoid-resistant small cell carcinoma cell line. *Journal of Endocrinology* **183** 375–383. (doi:10.1677/joe.1.05804)
- Weinstein RS, Jilka RL, Parfitt AM & Manolagas SC 1998 Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. *Journal of Clinical Investigation* **102** 274–282. (doi:10.1172/JCI2799)
- White A, Stewart MF, Farrell WE, Crosby SR, Lavender PM, Twentymen PR, Rees LH & Clark AJ 1989 Pro-opiomelanocortin gene expression and peptide secretion in human small cell lung cancer cell lines. *Journal of Molecular Endocrinology* **3** 65–70. (doi:10.1677/jme.0.0030065)
- Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM & Mak TW 1998 Apaf1 is required for mitochondrial pathways of apoptosis and brain development. *Cell* **94** 739–750. (doi:10.1016/S0092-8674(00)81733-X)

Received in final form 16 May 2011

Accepted 20 May 2011

Made available online as an Accepted Preprint

20 May 2011