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11β-Hydroxysteroid dehydrogenases, cell proliferation and malignancy☆

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Abstract

The enzymes 11β-hydroxysteroid dehydrogenase type 1 and 2 (11β-HSD1 and 2) have well-defined roles in the tissue-specific metabolism of glucocorticoids which underpin key endocrine mechanisms such as adipocyte differentiation (11β-HSD1) and mineralocorticoid action (11β-HSD2). However, in recent studies we have shown that the effects of 11β-HSD1 and 2 are not restricted to distinct tissue-specific hormonal functions. Studies of normal fetal and adult tissues, as well as their tumor equivalents, have shown a further dichotomy in 11β-HSD expression and activity. Specifically, most normal glucocorticoid receptor (GR)-rich tissues such as adipose tissue, bone, and pituitary cells express 11β-HSD1, whereas their fetal equivalents and tumors express 11β-HSD2. We have therefore postulated that the ability of 11β-HSD1 to generate cortisol acts as an autocrine anti-proliferative, pro-differentiation stimulus in normal adult tissues. In contrast, the cortisol-inactivating properties of 11β-HSD2 lead to pro-proliferative effects, particularly in tumors. This proposal is supported by experiments in vitro which have demonstrated divergent effects of 11β-HSD1 and 2 on cell proliferation. Current studies are aimed at (1) characterizing the underlying mechanisms for a 'switch' in 11β-HSD isozyme expression in tumors; (2) defining the molecular targets for glucocorticoids as regulators of cell proliferation; (3) evaluating the potential for targeting glucocorticoid metabolism as therapy for some cancers. These and other issues are discussed in the present review.

Keywords: 11β-Hydroxysteroid dehydrogenase; Glucocorticoid metabolism; Cell proliferation

1. Introduction

Glucocorticoids play an important role in normal physiology by modulating metabolic and immune responses. Circulating levels of glucocorticoids are sensitively maintained by the hypothalamic–pituitary–adrenal axis and, in common with other steroid hormones, they achieve their effects by binding to cognate intracellular receptors (glucocorticoid receptor (GR)), which then translocate to the nucleus. The resulting complex acts as a ligand-dependent *trans*activator by either binding as a homodimer to specific target gene response element (glucocorticoid response elements (GREs)), or by protein–protein interaction with other transcriptional regulators (Fig. 1). For example recent studies have shown physical interaction between GR and the p65 sub-unit of nuclear factor-κB (NF-κB). This appears to be central to the functional antagonism between NF-κB and glucocorticoid signalling pathways, which are characteristic of inflammatory and anti-inflammatory responses in the immune system [1]. Another important physical interaction occurs between the GR and the *fos-jun* sub-units of the ubiquitous *trans*activation protein-1 (AP-1). Once again, liganded GR complexes antagonize the pro-inflammatory activity of AP-1 but, as with NF-κB, this mechanism may have a more widespread impact on cell proliferation and differentiation.

In addition, 'prereceptor' regulatory mechanisms have been described for several steroid hormones that involve target tissue activation or inactivation of the circulating hormone [2]. For glucocorticoids, tissue-specific metabolism is catalyzed by two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD) [3], that interconvert biologically active cortisol and inactive cortisone. 11β-HSD1 acts predominantly as an oxoreductase in key glucocorticoid target tissues such as liver, gonads and adipose tissue, converting cortisone to cortisol, thereby regulating the level of active glucocorticoid available to the intracellular GR. In contrast, 11β-HSD2 is a dehydrogenase enzyme found predominantly in mineralocorticoid responsive tissues such as the kidney, salivary gland and colon. In these tissues 11β-HSD2 converts cortisol to cortisone, thereby protecting the mineralocorticoid receptor (MR) from inappropriate occupation by cortisol [3,4]. Thus, the two 11β-HSD isozymes act as critical regulators of glucocorticoid action at a tissue level, and their contribution

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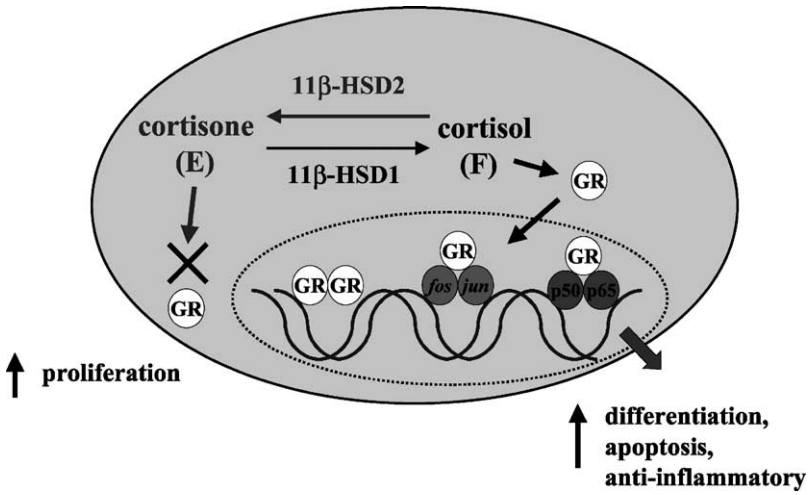

Fig. 1. 11β-HSD isozymes and the prereceptor regulation of GR-mediated *trans*activation. Interconversion of cortisol (F) and cortisone (E) catalysed by 11β-HSD1 and 2 determines the availability of ligand (F) for GR. Liganded GR modulates target gene transcription via specific GR response elements (GRE), or by interaction with NF-κB (p50/p65) or AP-1 (*fos-jun*). Glucocorticoid inactivation via the dehydrogenase activity of 11β-HSD2 decreases availability of active cortisol thereby facilitating proliferation. Reductase activity of 11β-HSD1 increases local cortisol levels and leads to increased differentiation and possibly apoptosis.

to classical endocrine diseases such as apparent mineralocorticoid excess [5] and central obesity [6,7] is well established.

2. 11β-HSD isozymes as determinants of cell proliferation

At a cellular level the actions of glucocorticoids are, at least in part, mediated via inhibition of cell proliferation and induction of differentiation. These responses, which have been demonstrated in a variety of tissues, are associated with glucocorticoid-mediated cell cycle arrest in the G_1-phase [8]. The precise mechanisms by which glucocorticoids regulate cell proliferation are poorly understood. However, amongst the most prominent glucocorticoid target genes are the cyclin-dependent kinases (CDKs) and their corresponding CDK inhibitors (CDIs) [9]. Some of these such as the Cip/Kip family of CDIs, particularly p57Kip2, are rapidly regulated by glucocorticoids and are central to the glucocorticoid-induced accumulation of cells in G_1-phase of

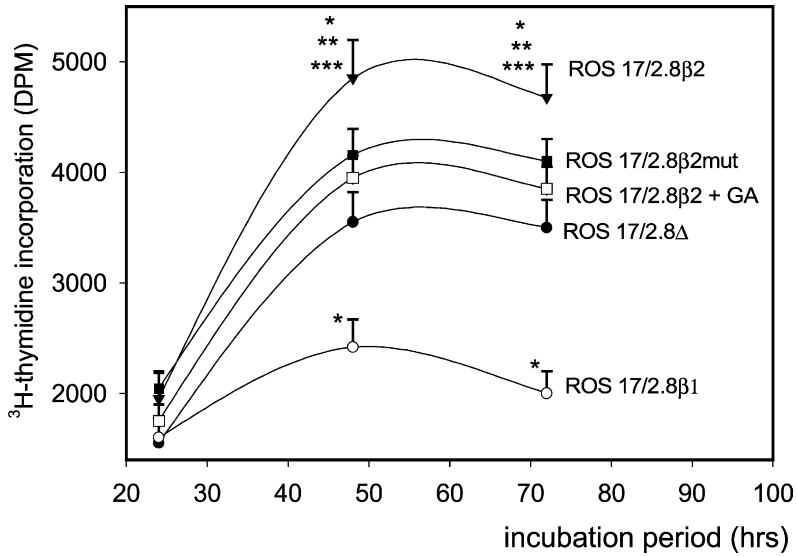

Fig. 2. Analysis of the growth kinetics of 11β-HSD isozyme transfectant cells. Plasmid only (ROS 17/2.8Δ) 11β-HSD1 (ROS 17/2.8β1), 11β-HSD2 (ROS 17/2.8β2), mutant 11β-HSD2 (ROS 17/2.8β2mut) and ROS 17/2.8β2 cells were treated with 10 μM 18β-glycyrrhetinic acid (GA) and cultured for 24, 48 and 72 h in basal 5% FCS-supplemented growth medium. Cell proliferation was assessed by analysis of nuclear ^{3}H-thymidine incorporation for the last 6 h of each incubation period. (*) Significantly different from ROS 17/2.8Δ cells, $P < 0.01$. (**) Significantly different from ROS 17/2.8β2 cells treated with 10 μM GA, $P < 0.05$. (***) Significantly different from ROS 17/2.8β2mut cells, $P < 0.05$ [18].

the cell cycle [8,10]. Abnormal expression of CDIs has been described in human pituitary tumors [11,12] and has also been implicated in the genesis of pituitary tumors in mice [13–15]. Interestingly, mice lacking both p18 and p27Kip1 rapidly develop fatal pituitary adenomas [16]. Glucocorticoids may also alter cell cycling by modulating growth factor-mediated changes in tyrosine kinase signalling, either by direct effects on membrane receptor expression or by indirect regulation of protein phosphorylation [17]. Furthermore, GR-mediated *trans*repression of NF-κ signalling is also important in controlling cell cycle progression [1].

In recent studies we have investigated novel effects of prereceptor regulation of glucocorticoid signalling, which have shown divergent effects of 11β-HSD1 and 2 on cell proliferation. Specifically, we have generated stable transfectant variants of the ROS 17/2.8 osteosarcoma cell line that over-express either human 11β-HSD1 or human 11β-HSD2 [18]. Cells expressing 11β-HSD1 showed lower rates of proliferation than vector-only controls, whereas cells expressing 11β-HSD2 showed much higher proliferation than controls (Fig. 2). The latter could be abrogated by addition of the HSD inhibitor glycyrrhetinic acid (GA), or by transfection of a non-functional mutant 11β-HSD2 cDNA. Crucially, the effects of 11β-HSD1 and 2 on cell proliferation were independent of any change in GR expression, further emphasising the pivotal role of these isozymes as determinants of cell proliferation. This indicated that the pro-proliferative effects of 11β-HSD2 were due to increased capacity for the local inactivation of cortisol (Fig. 2). The proliferative potential of 11β-HSD2 was further demonstrated by transient transfection studies using non-neoplastic HEK-293 human embryonic kidney cells that showed a 50% increase in proliferation 48 h after transfection with 11β-HSD2 cDNA [18]. Recent studies by other groups have also supported these conclusions. In this case targeted over-expression of 11β-HSD2, used as a means of disrupting glucocorticoid signalling in osteoblastic cells, was employed to block glucocorticoid-dependent responses in ROS 17/2.8 cells, including inhibition of cell proliferation [19].

3. Switching between 11β-HSD1 and 2

Although 11β-HSD1 and 2 are the products of separate genes and are expressed in distinct tissues, the concept that their actions are distinct and tissue-specific may no longer be true. Instead it seems likely that in several critical physiological and pathophysiological situations there is a 'switch' between the isozymes. Throughout early fetal development 11β-HSD2 is expressed in many tissues such as bone which normally express 11β-HSD1 in their adult equivalents [20]. Indeed, further recent studies by our group have shown that activity of 11β-HSD1 in bone actually increases with age [21]. This emphasises the differential patterns of expression for 11β-HSD1 and 2 and further supports the notion that the isozymes promote opposing patterns of cell proliferation.

In adult tissues there is a further 'switch' in glucocorticoid metabolism which occurs in the form of an alteration in the direction of 11β-HSD1 activity. Human ommental adipose stromal cells show a clear switch from 11β-HSD dehydrogenase to reductase activity during differentiation towards a mature adipocyte [22]. Although only 11β-HSD1 appears to be expressed, the switch in enzyme activity from glucocorticoid inactivating to glucocorticoid activating has the same net effect on proliferation, i.e. from pro-proliferation to anti-proliferation. This appears to be fundamental to stromal cell development. The third and most dramatic switch in 11β-HSD isozyme expression implicates 11β-HSD2 as a putative oncogene. Having highlighted a novel role for 11β-HSD1 and 2 as autocrine determinants of cell proliferation and differentiation, we have now postulated that inappropriate expression of 11β-HSD2 in GR-rich tissues may act as a pro-proliferative and potentially neoplastic stimulus to cell growth.

4. 11β-HSD isozyme expression in malignant tissue

Aberrant hormone signalling and action is a central feature in the pathogenesis of major cancers afflicting western populations, including breast cancer (estrogen, progesterone), prostate cancer (estrogen, testosterone), endometrial cancer (estrogen), colon cancer (estrogen, Vitamin D/lithocholic acid), and thyroid cancer (thyroid hormones, thyroid stimulating hormone) [23–25]. In breast, prostate and endometrial cancer the relationship between expression of cognate nuclear receptors (estrogen receptor (ER); androgen receptor (AR); progesterone receptor (PR)) and the local availability of ligand for these receptors has been shown to be crucial for tumor development [26–28]. Consequently, therapy for these cancers has successfully exploited both receptor modulators such as the ER antagonist tamoxifen [29] and inhibitors of aromatase [30] and 5α-reductase [23]. Furthermore, studies of non-classical steroid hormones such as retinoic acid, 1,25-dihydroxyvitamin D_3 and peroxisomal proliferator activated receptor (PPAR) ligands suggest that the metabolic and signal transduction pathways for these molecules may also provide potent targets for cancer therapy [31].

In contrast, the role of glucocorticoids in the pathophysiology and treatment of cancers is less clear. It is known that expression of the GR is almost universal for proliferating cells but GR function does not seem to be implicated in tumorigenesis. A splice-variant form of GR, GRβ, has been described which is almost identical to the original GR but does not bind ligand or activate transcription. Recent gene-reporter studies have suggested a dominant-negative role for GRβ, but the extent to which this is generally applicable in vivo is not yet clear [32,33]. The net effect of these observations is that, unlike the ER in breast cancer and the retinoic acid receptor in leukemias, it has been difficult to define a cancer lesion that is specifically associated with GR function. Thus, although GR-mediated mechanisms

have shown considerable potential in vitro, their use in vivo has achieved limited success [34]. By showing that differential regulation of 11β-HSD1 and 2 is a key determinant of cell proliferation, an intracrine mechanism independent of GR expression has been identified, that upon dysregulation could be implicated in tumorigenesis.

In normal aging tissues such as bone, a switch from 11β-HSD2 dehydrogenase activity in fetal tissue, to 11β-HSD1 reductase activity in adult tissues, has been described. In tumors this appears to reverse and tissues normally expressing 11β-HSD1 show expression of 11β-HSD2. An example of this situation is in the adrenal gland. In the human fetal adrenal gland 11β-HSD2 is detected whereas in normal adult adrenal glands 11β-HSD2 does not appear to be expressed. However, in adrenal cortical carcinoma and adenoma, 11β-HSD2 mRNA and protein are present [35]. Similar findings have been reported in breast tumor specimens [36] and colon cancer cells [37]. In addition, in vitro studies have described elevated 11β-HSD2 expression in leukemic [38] and MCF-7 and ZR-75-1 breast cancer cell lines [36,39]. In the latter, the high oxidative activity of 11β-HSD2 inactivated glucocorticoids and thereby abrogated their anti-proliferative action. However, addition of an 11β-HSD2 inhibitor blocked glucocorticoid inactivation and resulted in restoration of the anti-proliferative effect, causing a 40–47% decrease in cell proliferation. Furthermore, recent microarray analyses have shown significant over-expression of 11β-HSD2 in breast and prostate tumors [40,41]. Studies in primary bone cell cultures have revealed exclusive expression of 11β-HSD1, although in osteosarcoma cell lines, high levels of 11β-HSD2 expression were apparent [42–44]. Other reports describing 11β-HSD isozyme activity in Ishikawa cells, an endometrial cancer cell line, have confirmed the anti-proliferative action of glucocorticoids but more significantly showed that inhibition of 11β-HSD2 activity by glycyrrhetinic acid enhanced the anti-proliferative effects of glucocorticoids [45]. In each of these cases, the upregulation of 11β-HSD2 in neoplastic tissue and cell lines contrasts with their normal tissue equivalents (Table 1). Significantly, the presence of 11β-HSD2 in many of these systems is associated with GR rather than MR expression suggesting an alternative function for the isozyme that is distinct from its classical role in MR-rich tissues such as the colon or kidney. This would have a profound effect on cellular function considering the ubiquitous expression of the GR and its fundamental role in anti-proliferative signalling.

Table 1
Summary of 11β-HSD isozyme expression in neoplastic tissues and cell lines

Study model	11β-HSD1 expression	11β-HSD2 expression	Reference
Osteosarcoma cell lines (TE-85, MG-63, SaOS-2)	×	✓	[42,43]
Fibrosarcoma cell line (Hs913)	×	✓	[42]
Endometrial cancer cell line (Ishikawa)	×	✓	[45]
Breast cancer cell lines (MCF-7, ZR-75-1, PMC42)	×	✓	[36,39]
Myelomonocytic cell line (U937)	×	✓	[38]
Adrenal cortical carcinoma/ adenoma	×	✓	[35]
Colon carcinoma	×	✓	[64]
Colon carcinoma cell lines (Caco-2, Ht-29)	×	✓	[37]
Breast tumor specimens	×	✓	[36]
Pituitary adenomas	×	✓	[49]
Squamous cell carcinomas of the head and neck	↓		[65]

5. 11β-HSD in the pituitary

Perhaps the most conclusive evidence for the role of 11β-HSD2 in tumor development has come from the analysis of 11β-HSD isozyme expression in a large cohort of human pituitary tumors. While the expression of 11β-HSD isozymes in both rodent and human pituitaries has been well documented [46–48], the function of 11β-HSD in the pituitary has yet to be fully clarified although it seems unlikely to represent an additional level of endocrine regulation within the established hypothalamic–pituitary–adrenal axis [46]. Using quantitative real-time PCR a marked difference was observed between normal and tumorous pituitaries with respect to 11β-HSD2 expression. 11β-HSD1 expression was significantly reduced (0.2-fold) while expression of 11β-HSD2 was increased (approximately 10-fold) in pituitary tumors compared with normals. Moreover, enzyme conversion data also showed that 11β-HSD activity in pituitary adenomas was exclusively due to the type 2 isozyme. Inhibition of 11β-HSD2 by glycyrrhetinic acid inhibited pituitary cell proliferation and also potentiated the anti-proliferative effect of exogenously added cortisol [49].

It appears from this model that a switch in 11β-HSD isozyme expression in the pituitary may confer a growth advantage and thus contribute to the process of pituitary tumorigenesis. Reduced 11β-HSD1 in pituitary tumors is likely to result in attenuated local conversion of cortisone to cortisol. Moreover, significantly increased 11β-HSD2 expression in pituitary tumors will further reduce the availability of biologically active cortisol by enhancing its inactivation to cortisone. Thus the net effect of the observed switch in 11β-HSD isozyme expression in pituitary tumors is to reduce availability of active, anti-proliferative glucocorticoids. This highlights the role of 11β-HSD2 as a prereceptor regulator of pituitary cell growth and potentially uncovers a novel tumor marker that may help to elucidate fundamental aspects of the pathophysiology of the pituitary and other neoplasms.

6. Future developments

Analysis of the association between 11β-HSD isozymes and malignancy has raised several important questions.

Firstly, the key question remains as to what is the likely mechanism leading to decreased expression of 11β-HSD1 and increased expression of 11β-HSD2 in tumors. Analysis of the promoter regions of the genes for the two enzymes reveals some potential candidate target sequences. Prominent amongst these in the 11β-HSD1 gene (HSD11B1) are binding sites for NF-κB, CCAAT/enhancer binding protein (C/EBP), PPARγ, as well as GREs. Current data using the adipose stromal cell model suggest that cortisol itself is the most potent activator of HSD11B1 expression, which may reflect the presence of GRE within the promoter. However, in previous studies we have shown that 11β-HSD1 activity in primary human adipocytes is stimulated by inflammatory cytokines and the natural PPARγ ligand 15-deoxy-12,14-PGJ2 [50]. In contrast, reports from other groups using mouse 3T3-L1 adipocytes suggest that synthetic PPARγ agonists inhibit 11β-HSD1 expression [51]. The only reported promoter analysis for 11β-HSD1 utilizing the rat gene promoter has highlighted C/EBPα as a key activator of 11β-HSD1 in the liver, while C/EBPβ antagonized the effect of C/EBPα [52]. Furthermore, mice with C/EBPα gene ablation showed lower expression of 11β-HSD1 whereas C/EBPβ knockout increased the enzyme [52]. C/EBPs are clearly linked to energy metabolism with knockout mice dying as a consequence of hypoglycaemia [53]. However, they also play an important role in the regulation of cell growth and differentiation. C/EBPα is essential for proper lung and liver function and granulocytic and adipose tissue differentiation and mutation of this transcription factor is associated with some malignancies, notably acute myeloid leukemias [54]. Over-expression of C/EBPα in established cell lines inhibits cell proliferation and decreased C/EBPα expression has been shown to be associated with increased DNA synthesis [55] and liver carcinomas [56]. Thus it is tempting to compare the effects of C/EBPα knockout with the changes in proliferation and tumor expression we have reported with decreased expression of 11β-HSD1 [41,44,46].

PPARγ is another potentially important regulator of 11β-HSD1, particularly with regard to adipocyte development, although variable effects of PPARγ agonists on glucocorticoid metabolism have been reported [50,51]. C/EBPα but not C/EBPβ has been shown to increase PPARγ2 expression [57] highlighting a close association between the two factors. Significantly a variety of recent studies have highlighted the importance of PPARγ both as a marker of tumor prognosis and as a therapeutic target through the use of thiazolidinedione (TZD) receptor agonists. Perhaps the most significant example of this are the recent studies by Heaney and colleagues who demonstrated high levels of PPARγ in ACTH-secreting pituitary tumors as well as inhibition of murine ACTH tumors using the PPARγ agonist rosiglitazone [58].

Sequence analysis of 11β-HSD2 mRNAs from normal tissue (placenta) and an equivalent tumor cell line (JEG-3) has revealed identical 5′ sequences, suggesting that tumor expression of 11β-HSD2 is not due to alternative use of upstream exons (unpublished data). Other studies have mapped out the promoter motifs associated with tissue-specific expression of 11β-HSD2 [59]. In particular they highlighted the importance of a series of Sp1 and NF-κB sites within the proximal promoter (−210 to –45 bp) in determining basal transcription. However, an important limitation with this study was that the in vivo footprinting and promoter-reporter data were derived from tumor cell lines and not primary cultures from normal tissue. Thus the *cis*-acting elements identified in this study may be more important for attenuation of 11β-HSD2 activity in tumors; for example we have shown that NF-κB activators are potent inhibitors of 11β-HSD2 expression and activity in osteosarcoma cells [60]. Further studies to identify the key factors associated with induction of 11β-HSD2 expression are currently ongoing within our group.

The expression of 11β-HSD2 in tumors may also possibly provide a novel therapeutic approach to the treatment of cancer. Previous reports have described the anti-proliferative properties of the liquorice derivatives glycyrrhizin and glycyrrhetinic acid [61,62]. Glycyrrhizin is one of the key ingredients in the Japanese herbal medicine “Sho-saiko-to”, which has been used for centuries in the Far East for its potent anti-cancer properties [61]. However, liquorice derivatives also have potent effects on 11β-HSD2 in classical tissues such as the kidney [3,63]. As such they are able to reproduce the symptoms of Apparent Mineralocorticoid Excess leading in turn to abnormal sodium retention and increased blood pressure. Further analysis of the kinetics of these and other 11β-HSD inhibitors may help to determine whether or not 11β-HSD2 will provide a suitable target for specific therapeutic intervention in the treatment of cancer. Alternatively, it is possible that tissue-specific introduction of 11β-HSD1 to tumors using gene therapy may abrogate the deleterious effects of 11β-HSD2. Further characterization of the expression and regulation of 11β-HSD isozymes in cancer may help to answer these questions but will also shed further light on the fundamental role of glucocorticoids in tumor development and treatment.

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