Cellular and Molecular Life Sciences (2019) 76:13–26
https://doi.org/10.1007/s00018-018-2918-5

REVIEW

Mechanisms for establishment of the placental glucocorticoid barrier, a guard for life

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Received: 5 June 2018 / Revised: 16 August 2018 / Accepted: 6 September 2018 / Published online: 17 September 2018

Abstract

The fetus is shielded from the adverse effects of excessive maternal glucocorticoids by 11β-HSD2, an enzyme which is expressed in the syncytial layer of the placental villi and is capable of converting biologically active cortisol into inactive cortisone. Impairment of this placental glucocorticoid barrier is associated with fetal intrauterine growth restriction (IUGR) and development of chronic diseases in later life. Ontogeny studies show that the expression of 11β-HSD2 is initiated at a very early stage after conception and increases with gestational age but declines around term. The promoter for *HSD11B2,* the gene encoding 11β-HSD2, has a highly GC-rich core. However, the pattern of methylation on *HSD11B2* may have already been set up in the blastocyst when the trophoblast identity is committed. Instead, hCG-initiated signals appear to be responsible for the upsurge of 11β-HSD2 expression during trophoblast syncytialization. By activating the cAMP/PKA pathway, hCG not only alters the modification of histones but also increases the expression of Sp1 which activates the transcription of *HSD11B2.* Adverse conditions such as stress, hypoxia and nutritional restriction can cause IUGR of the fetus. It appears that different causes of IUGR may attenuate *HSD11B2* expression differentially in the placenta. While stress and nutritional restriction may reduce *HSD11B2* expression by increasing its methylation, hypoxia may decrease *HSD11B2* expression via alternative mechanisms rather than by methylation. Herein, we summarize the advances in the study of mechanisms underlying the establishment of the placental glucocorticoid barrier and the attenuation of this barrier by adverse conditions during pregnancy.

Keywords Placenta · Cortisol · 11β-hydroxysteroid dehydrogenase 2 · Ontogeny · hCG · Histone and epigenetics

Introduction

Glucocorticoids, the end products of the hypothalamus–pituitary–adrenal (HPA) axis, are released in response to stress to regulate a variety of vital functions thereby maintaining homeostasis [1]. In many species, including amphibians, reptiles, rodents and birds, corticosterone is a major biologically active glucocorticoid, while in humans, cortisol is the primary biologically active glucocorticoid. Compelling evidence indicates that glucocorticoids are also important in reproduction [2] and they are not only crucial to the establishment of pregnancy [3], but also pivotal in fetal development [4, 5] and parturition [6, 7]. With regards to fetal development, glucocorticoids act as a double-edged sword depending on exposure time and levels [8]. Towards the end of gestation, appropriate exposure to glucocorticoids are necessary for the maturation of a number of fetal organ systems, particularly the respiratory and digestive systems, to prepare the fetus for extra-uterine life. Thus, synthetic glucocorticoids are widely used in pregnant women with threatened preterm delivery to improve neonatal viability by accelerating lung maturation [5, 9]. Despite of these beneficial effects, excessive glucocorticoid exposure is known to exert a number of adverse effects on the fetus. Accumulating evidence indicates that over-exposure of the fetus to glucocorticoids not only causes fetal growth restriction but also programs the development of chronic diseases such as

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hypertension, insulin resistance and behavioral abnormalities with the possibility of altered activity of the HAP axis in later life [8, 10]. Therefore, it is essential to control glucocorticoids at optimal levels in the fetal circulation to ensure a safe intrauterine development.

The fetus is shielded by a placental glucocorticoid barrier

The development of the primate fetal adrenal glands is a unique process. The fetal adrenal glands are relative large in comparison with the adult organs. A large fetal zone dominates the cortex throughout gestation. This fetal zone produces dehydroepiandrosterone (DHEA) and its sulfate instead of cortisol [11, 12]. Although a small amount of cortisol can be synthesized from progesterone in the fetal adrenal glands [13], limited de novo cortisol synthesis from cholesterol is not established until the third trimester of pregnancy in the fetal adrenals [11]. In contrast, the maternal adrenal glands gradually become hypertrophic during pregnancy [14]. Although the overall weight and size do not change dramatically, the size of the zona fasciculata which produces cortisol is increased [14]. During pregnancy, there is a three- to eightfold increase in the levels of total cortisol in the maternal circulation [14]. Despite of increased synthesis of corticosteroid binding protein (CBG) by the liver in gestation [15], a two- to fourfold elevation of free cortisol in maternal circulation is still seen from the second–third trimesters [16–18]. Therefore, the amount of cortisol produced by the fetal adrenal glands is minimal compared with that produced by the maternal adrenal glands. However, meet the requirement for fetal organ maturation towards the end of gestation, it is still necessary for the fetus to acquire a sufficient proportion of cortisol from the maternal side. It is estimated that about 40–50% of fetal cortisol is derived from the mother towards the end of gestation [19]. Nonetheless, the majority of maternal cortisol is still blocked from the fetus, despite its lipophilic nature to create a safe environment for development of the fetus since excessive glucocorticoids are detrimental to the fetal development [8, 10]. It is estimated that there is only 15% of maternal cortisol crossing the placenta unmetabolized in normal pregnancy [20].

It is very well recognized that biologically active cortisol or corticosterone is converted into biologically inactive cortisone or 11-dehydrocorticosterone when passing through the placenta in almost all studied placental mammals [20, 21]. It has been reported that the conversion of cortisol into cortisone by homogenized human placental tissue dominates at all gestational ages, albeit a small amount of cortisone can also be converted into cortisol [22]. As such, cortisol levels in the fetal circulation are kept about tenfold lower than those in the maternal circulation [14, 19].

11β-HSD2 acts as the placental glucocorticoid barrier

It is now widely accepted that the glucocorticoid inactivating enzyme, 11β-hydroxysteroid dehydrogenase2 (11β-HSD2), acts as the placental glucocorticoid barrier [20, 23–25]. In addition to 11β-HSD2, a glucocorticoid regenerating enzyme 11β-HSD1 [25, 26] has also been identified. These two glucocorticoid metabolizing enzymes work in opposing ways with differential affinities for their substrates. While 11β-HSD1 is a reductase converting biologically inactive cortisone or 11-dehydrocorticosterone into active cortisol or corticosterone with a Km value in the micromolar range and requiring NADPH as its cofactor, 11β-HSD2 is an exclusive oxidase converting biologically active cortisol or corticosterone into inactive cortisone or 11-dehydrocorticosterone with a Km value in the nanomolar range and requiring NAD^+ as its cofactor [25–30].

Table 1 summarizes the main characteristics of 11β-HSD1 and 11β-HSD2. Examination of their distribution in the body revealed that the reductase 11β-HSD1 is distributed widely in the glucocorticoid-target tissues in the body, while the oxidase 11β-HSD2 is distributed mainly in the mineralocorticoid-target organs including the kidney, intestine, salivary glands, exocrine pancreatic gland and sweat glands [25, 26, 29, 30]. The distribution patterns of 11β-HSD1 and 11β-HSD2 are in line with the distribution of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) in these tissues, respectively. Cortisol binds to GR with a relatively low affinity (Kd: 11 nM), while the MR has equal and high affinities for both cortisol and aldosterone (Kd: 0.5 nM) [31]. The reductase activity of 11β-HSD1 can create more biologically active glucocorticoids in glucocorticoid-target cells, so that adequate amounts of glucocorticoids can be obtained for the low-affinity GR. Therefore, 11β-HSD1 is regarded as a pre-GR amplifier for glucocorticoid actions [32]. Likewise, the specific distribution of 11β-HSD2 in the mineralocorticoid target organs also has its own designated function. Since the MR is bound by cortisol and aldosterone with similar affinities, it is necessary for the mineralocorticoid target cells to express 11β-HSD2 to inactivate cortisol, the concentration of which is about 100- to 1000-fold higher than that of aldosterone in the circulation. This ensures the specificity of MR for aldosterone. Otherwise, the MR would be occupied by the overwhelming glucocorticoid concentration resulting in severe sodium and water retention [31, 33].

The placenta is generally considered a non-classical mineralocorticoid-target tissue, despite the presence of MR in human placenta [34]. Nonetheless, the placenta expresses abundant 11β-HSD2 [25, 35, 36], which is

Table 1 Characteristics of 11β-HSD1 and 11β-HSD2 in humans

	11β-HSD1	11β-HSD2
Gene	*HSD11B1*	*HSD11B2*
Chromosome	1q32	16q22
Number of exons	6	5
Promoter	Consensus CAAT box, no TATA box	Rich in CpG, no TATA box
Molecular weight	34 kD	44 kD
Number of amino acids	292	406
Reaction type	Reductase	Oxidase
Co-factor	NADPH	NAD^+
Affinity	Low (Km: μM)	High (Km: nM)
Substrate	Cortisone	Cortisol
Product	Cortisol	Cortisone
Effect on glucocorticoids	Amplification	Inactivation
Distribution in the body	Glucocorticoid-target tissues	Mineralocorticoid-target tissues
Co-localization	Glucocorticoid receptor	Mineralocorticoid receptor
Distribution in the intrauterine tissues	Amnion and chorion of the fetal membranes; stromal and epithelial cells of the decidua, endothelial cells of the fetal blood vessels in the villous core	Placental villous syncytiotrophoblast, decidual epithelial cells

believed to function mainly as a barrier for maternal glucocorticoids [37], although alternative functions such as regulating the local actions of glucocorticoids in the placenta have also been suggested [38]. In addition, the high affinity of 11β-HSD2 for cortisol makes it more suited to serve as a placental glucocorticoid barrier guarding the fetus against maternal glucocorticoids [24, 28].

Distribution and ontogenesis of 11β-HSD2 in the placenta

Compartmentalized distribution of 11β-HSD2 at the fetal-maternal interface and its implications

The human placenta is classified as hemochorial. This type of placenta is characterized by the direct contact of the maternal blood circulation with the placental villi where the maternofetal nutrients and gas exchanges take place. All villi are covered by two layers of trophoblasts. The outermost layer is the terminally differentiated and continuous multinucleated syncytiotrophoblast, and the inner layer is composed of single and aggregated cytotrophoblasts which are highly proliferative and can differentiate into either villous syncytiotrophoblast or extravillous invasive trophoblasts in the processes of placentation and implantation. Since the syncytiotrophoblast layer lines the intervillous space, it provides the first line of defense for the fetus against any potentially harmful substances from the maternal side.

Prior to the acknowledgement of two types of 11β-HSDs in 1990s [25, 26], it was known for around three decades that the human placenta is capable of oxidizing biologically active 11β-hydroxycorticosteroids into inactive 11-oxo-compounds [39]. As described above, the human placenta maintains a high oxidase activity at all gestational ages [20, 40–42], albeit a minimal increase in the reductase activity with increasing gestational age in the placenta [21, 22]. Interestingly, in contrast to the dominant oxidase activity in the placenta, a reductase activity of 11β-HSD has been demonstrated to be dominant in the human fetal membranes [42–44]. Immunohistological staining of placental sections at both term and preterm revealed compartmentalized patterns of 11β-HSD1 and 2 with distinct distributions [25, 35, 45–48]. Specifically, 11β-HSD2 is exclusively localized to the syncytiotrophoblast of the chorionic villi but not to the extravillous trophoblasts, villous cytotrophoblasts and villous core [25, 35, 45–48] (Fig. 1). The absence of 11β-HSD2 in the extravillous trophoblasts suggests that glucocorticoids are required for the invasion of extravillous trophoblasts into the endometrium during implantation.

Several studies have shown that glucocorticoids are implicated in a number of events pertinent to the establishment of implantation including inhibition of the maternal immune intolerance of the semi-allograft embryo, enhancement of endometrial stromal cell decidualization and stimulation of human chorionic gonadotropin (hCG) production by the trophoblasts [3]. The specific distribution of 11β-HSD2 in the outer continuous syncytial layer of the chorionic villi can be traced back to as early as the 3rd week post-conception [46, 47], when remodeling of maternal spiral arteries and blood supply to the placenta is fully accomplished and the process of placentation is completed [49]. This early

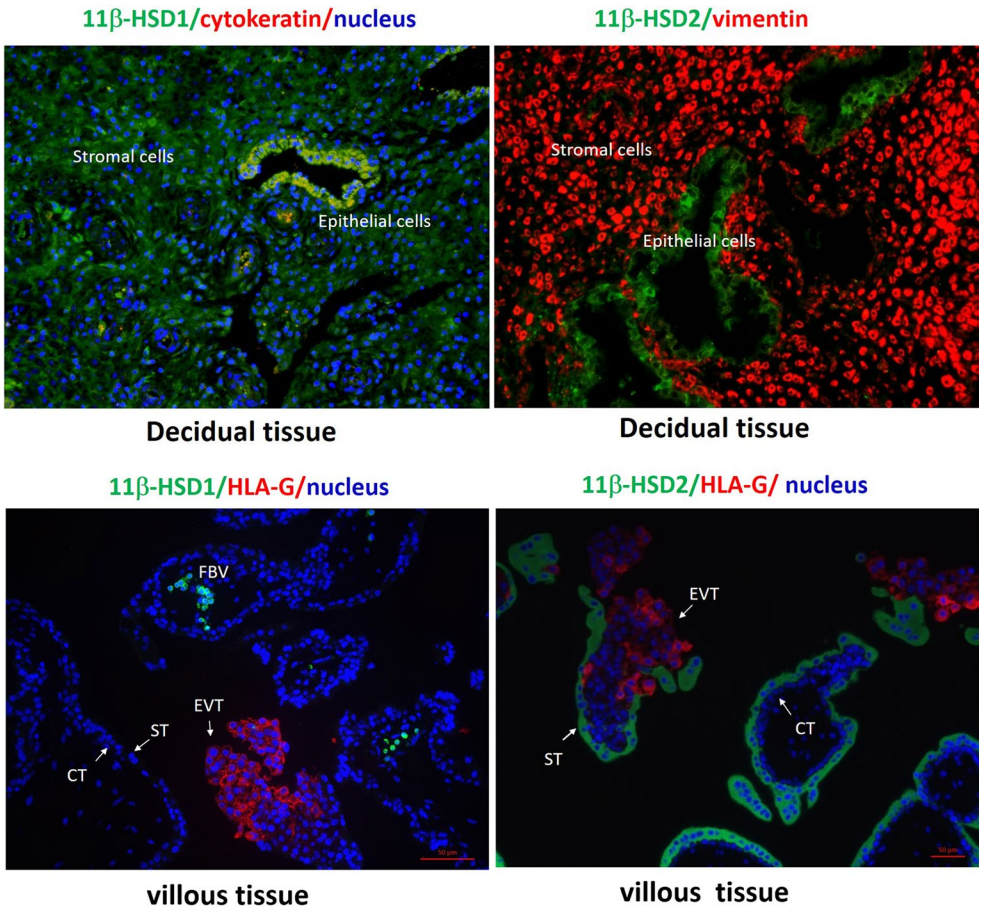


Fig. 1 Distribution of 11β-HSD1 and 2 in human decidua and chorionic villous tissues during early gestation. Top panel: Decidual epithelial cells are cytokeratin 7 positive and stromal cells are vimentin positive. Bottom panel: Villous trophoblasts are HLA-G negative while extravillous trophoblasts are HLA-G positive. *CT* cytotrophoblast; *ST* syncytiotrophoblast; *EVT* extravillous trophoblast; *FBV* fetal blood vessels. Based on the work presented in the Ref. [46]. Human tissue collection was approved by the Ethics Committee of Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine with informed consent

appearance of 11β-HSD2 on the surface of chorionic villi suggests a critical role of 11β-HSD2 in the protection of embryo development even at this very early stage of gestation. Unfortunately, it is unclear whether the expression of 11β-HSD2 is already initiated in the trophoblasts of the trophectoderm of the implanting embryo. If so, the shield against maternal glucocorticoids might already be established even before the completion of placentation. Notably, the formation of the trophoblast plug in the erosive spiral artery can prevent the implanting embryo from direct maternal blood flushing and this plug is not removed until the end of the 6th week after conception [50]. Nonetheless, the implanting embryo is still immersed and nourished by secretions from the endometrial cells [51], which may contain relatively high concentrations of glucocorticoids. Our immunohistochemical staining of the first trimester endometrium shows that abundant 11β-HSD1 is present in the endometrial stromal cells, glandular and endometrial epithelium [46] (Fig. 1), which suggests that, on the one hand, cortisol regeneration is required for the implantation, and that, at the same time, cortisol regeneration may heighten the concentration of cortisol in the endometrial secretion, thus imposing threats to the developing embryo. Therefore, it is very likely that 11β-HSD2 expression is already set up in the trophectoderm layer for the protection of the implanting embryo.

Ontogeny of 11β-HSD2 expression in the placenta

Examination of the ontogeny of placental 11β-HSD2 expression reveals that 11β-HSD2 mRNA and activity increase with gestational age until late gestation [52–55]. McTernan et al. reported 12- and 56-fold increases in 11β-HSD2 mRNA abundance in the villous tissue by early third trimester (27–34 weeks) and term, respectively, in comparison with the levels observed at 4–6 weeks after conception [52]. Schoof et al. reported similar increases in 11β-HSD2 mRNA abundance in the placenta from 16 to 40 weeks of gestation [53]. In addition, Murphy et al. found that there was no further change in 11β-HSD2 mRNA abundance in the placenta around term (36, 37, 38 and > 38 weeks) [56].

11β-HSD2 activity studies reveal a similar pattern of increases across gestational age [54, 55] but there also appeared to be a decline after 36 weeks [56]. Similar declines are observed close to term in other animal species as well, including rats [57], mice [58–60], rabbits [61] and guinea pigs [62]. Therefore, a decline in 11β-HSD2 activity in the placenta around term may be a generalized

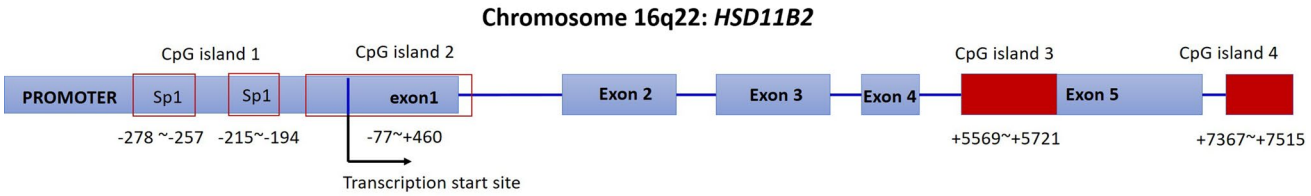
phenomenon across species and it indicates the requirement of maternal glucocorticoids for fetal organ maturation as well as parturition. In addition, the presence of 11β-HSD1 in the endothelium of the fetal blood vessels in the villous core and in the umbilical cord [63] may also assist in the acquisition of maternal glucocorticoids for this purpose. Indeed, a placental perfusion study shows that a considerable amount of cortisone perfused into the intervillous space on the maternal side is converted into cortisol when passing through the placenta into the fetal circulation [21].

Pathways signaling the establishment of the placental glucocorticoid barrier

Does DNA methylation matter in the setup of placental glucocorticoid barrier?

As described above, 11β-HSD2 exhibits cell-specific expression in mineralocorticoid target cells. This cell-specific expression of 11β-HSD2 is now known to be attributed to the epigenetic modification of *HSD11B2*, the gene encoding 11β-HSD2. The human *HSD11B2* gene, which is localized to chromosome 16q22, consists of five exons spreading across 6.2 kb which are separated from each other by small introns [64]. RNase protection assays identified the presence of tissue-specific transcriptional start sites. In the placenta, transcription starts mainly at −74 nucleotides (nt) and to a lesser extent at −116 nt. In contrast, the kidney utilizes the −116 nt almost exclusively [65]. The promoter of the human *HSD11B2* gene lacks a TATA box but has a highly enriched GC core, suggesting that the gene may be regulated transcriptionally by factors such as Sp1 which recognize GC-rich sequences [65].

Using luciferase reporter constructs, Agarwal et al. identified that the region from −2 to −330 nt relative to the initial ATG codon was an essential region for the basal transcription of the *HSD11B2* gene [65]. Foot printing or gel shift experiments show that this region contains two GC-rich domains (−278 to −257 nt and −215 to −194 nt) which harbors the canonical binding sites for Sp1 [65]. Apart from the CpG island in the promoter, another three CpG islands are found spanning the promoter and exon 1 (−77 to +460 nt), in exon 5 (+5569 to +5721 nt) and in the downstream region (+7367 to +7515 nt) [66] (Fig. 2). Of these four CpG islands, the two downstream ones are fully methylated in either mineralocorticoid or non-mineralocorticoid target tissues, while the methylation levels of the CpG island in the promoter region are low in mineralocorticoid target tissues, but high in non-mineralocorticoid target tissues [66] (Fig. 2). Of note, the CpG island spanning the promoter and exon 1 is only slightly methylated in both mineralocorticoid or non-mineralocorticoid target tissues [66] (Fig. 2). These data suggest that the methylation level of the CpG island in the promoter is a determining factor as to whether the expression of *HSD11B2* is repressed or not, and hypermethylation of this CpG island is responsible for the muted expression in non-mineralocorticoid-target cells [66].



Methylation levels	mineralocorticoid target tissue	non-mineralocorticoid target tissue	Placenta tissue
CpG island 1	low	high	low
CpG island 2	low	low	low
CpG island 3	high	high	high
CpG island 4	high	high	high

Fig. 2 Locations of the four CpG islands in *HSD11B2* gene and the methylation levels in mineralocorticoid and non-mineralocorticoid target tissues, and in cytotrophoblast and syncytiotrophoblast. Based on the data presented in references 25 and 66. Boxes in red either filled or blank are indications of CpG islands

Of interest, Alikhani-Koopaei et al. found that the methylation levels of the CpG island in *HSD11B2* promoter were low in human placental tissue. Consistent with this, a recent study by Hu et al. also reported that almost no 11β-HSD2 methylation was detected in both normal and preeclamptic placental tissues [67]. These findings not only explain why the placental syncytiotrophoblast can maintain such high levels of 11β-HSD2 expression, but also raise an issue as to why 11β-HSD2 is barely expressed in the cytotrophoblasts [46, 47, 63], the progenitor cells for the syncytiotrophoblast. Can DNA methylation be excluded from the mechanisms underlying the silencing of 11β-HSD2 expression before syncytialization?

DNA methylation plays a crucial role in defining cell fate in mammalian development, which provides an epigenetic barrier that reduces developmental potential of a particular cell while helping to establish its distinct cellular identity. The DNA methylation marks in the parental gametes are erased on a global scale in the zygote immediately following fertilization to restore the developmental totipotency [68]. The re-establishment of DNA methylation marks starts during the segregation of the inner cell mass and trophectoderm of the embryo with the commitment towards a distinct cell fate [68, 69]. While the inner cell mass eventually gives rise to the definitive structures of the fetus, the trophectoderm gives rise to the placenta including the cytotrophoblasts, the progenitor cells for both extravillous trophoblasts and villous syncytiotrophoblast. Because low methylation levels of the CpG island in the *HSD11B2* promoter are detected in the placenta tissue [66], it is conceivable that the low methylation levels of the CpG island in the *HSD11B2* promoter is already set up when the trophoblast fate is determined during the segregation of the trophectoderm and inner cell mass. We compared the methylation levels of the CpG domain (− 244 to + 16) in the *HSD11B2* promoter in human placental cytotrophoblasts and syncytiotrophoblasts, and found no differences in the methylation levels in this region between these two types of cells [70]. Although no difference in the methylation level of the CpG island in the *HSD11B2* promoter was found between cytotrophoblasts and syncytiotrophoblasts, sporadic methylated cystines were nevertheless observed in this region in both cytotrophoblasts and syncytiotrophoblasts [70]. Currently, we are not clear about the meaning of these sporadic methylations in the regulation of *HSD11B2* expression in the trophoblasts.

Our data support the fact that the methylation pattern of the *HSD11B2* promoter is already established when the cytotrophoblast fate is determined in the trophectoderm. If it is not a matter of methylation, the question remains as to what is the signaling pathway that underlies the upsurge of *HSD11B2* expression during syncytialization. Our studies demonstrate that histone modification and transcription factors activated by hCG during syncytialization might play a critical role in this process [45, 48, 70].

Role of hCG-activated signaling pathways in the setup of the placental glucocorticoid barrier

HCG is the first hormone produced by the trophoblasts of the implanting blastocyst. There are two major forms of hCG in pregnancy: the classical and hyperglycosylated hCG. The classical hCG is produced primarily by differentiated syncytiotrophoblasts, while the hyperglycosylated hCG is produced by the extravillous trophoblasts [71, 72]. The hyperglycosylated hCG is believed to drive the invasion of extravillous trophoblasts into the myometrium possibly through the TGFβ receptor thereby assisting in the implantation process. The best characterized role for the classical hCG is to maintain the function of corpus luteum for the production progesterone and estrogen through the cAMP/PKA pathway coupled with the hCG/LH receptor until the steroidogenic activity of the placenta is fully established around the 3rd and 4th weeks of gestation [72]. Intriguingly, the secretion of hCG continues to increase even after this mission is accomplished and peaks of hCG production are observed around the 10th week of gestation. The concentration of hCG declines after this, but it is still maintained at a high level which is about 18% of the peak value [72]. This pattern of hCG production in gestation strongly indicates a role of hCG beyond the mere regulation of implantation and maintenance of corpus luteum functions in early gestation. Accumulating evidence indicates that hCG is involved in multiple functions that maintain gestation, including promoting angiogenesis in the uterine endothelium, maintaining myometrial quiescence and enhancing syncytialization, a process that continues throughout pregnancy [72, 73].

Our study indicates that hCG is also essential in the upsurge of *HSD11B2* expression during syncytialization [74]. We found that hCG reduced the phosphorylation of retinoblastoma protein (pRB) via activation of the cAMP/PKA pathway, which sequesters E2F transcription factor 1 (E2F1). This is the transcription factor for EZH2 expression which results in the inactivation of the pRB–E2F1–EZH2 pathway and removal of the repressive marker trimethylation of histone H3 lysine 27 (H3K27me3) at the *HSD11B2* promoter [48] (Fig. 3). The removal of H3K27me3 renders H3K27 available for acetylation ($H3K27_{Ac}$) by p300, which transforms the chromatin from a compact structure into a loose form, allowing access of the HSD11B2 promoter to the transcription factor, Sp1, which enhances its transcription [48] (Fig. 3). Consistently, the hCG-activated cAMP pathway during syncytialization causes a dramatic decrease in EZH2 expression in addition to the reduction in pRB

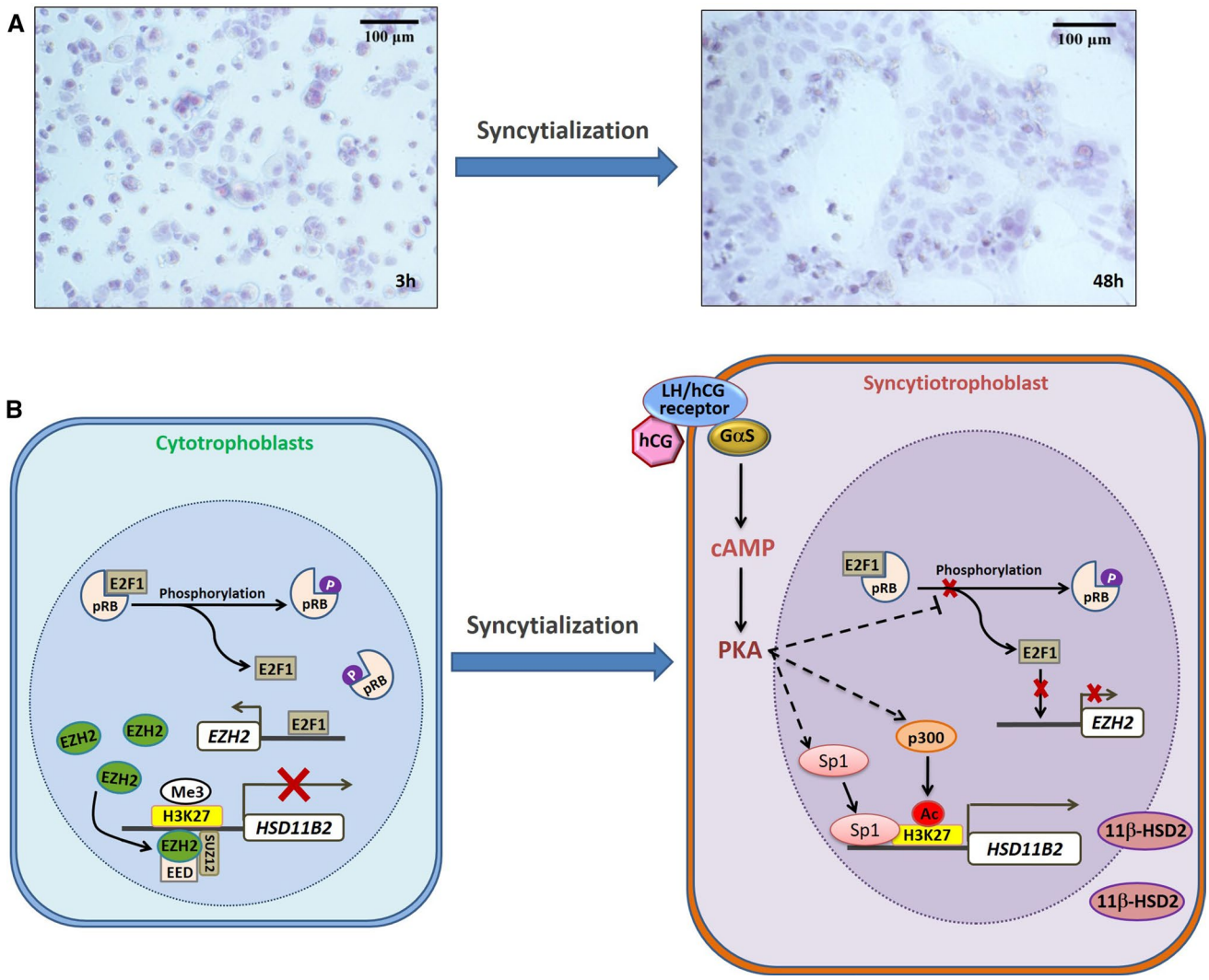


Fig. 3 Diagram illustrating the regulation of *HSD11B2* expression by human chorionic gonadotropin (hCG)-triggered cAMP/PKA pathway during syncytialization of the trophoblasts. Panel A shows the syncytialization of human primary placental trophoblasts under cultured conditions in DMEM containing 10% fetal calf serum. Panel B illustrates the mechanism underpinning the upregulation of *HSD11B2* expression during syncytialization. Before syncytialization, phosphorylation of retinoblastoma protein (pRB) frees the E2F transcription factor 1(E2F1) which drives the expression of the enhancer of zeste homolog 2 (EZH2) in cytotrophoblasts. Subsequently, EZH2 together with cofactors embryonic ectoderm development (EED) and suppressor of zeste 12 homolog (SUZ12) cause trimethylation (Me3) of histone H3 lysine 27 (H3K27) associated with *HSD11B2* promoter resulting in diminished expression of *HSD11B2*. Upon syncytialization, hCG is produced in large quantities and activates the cAMP/PKA pathway, which indirectly blocks the phosphorylation of pRB. Dephosphorylation of pRB sequesters E2F1 thereby reducing the expression of EZH2 and resulting in the attenuation of the down-stream H3K27 methylation. Attenuated H3K27 methylation predisposes H3K27 to acetylation (Ac) by p300, which transforms the condensed chromatin into a more relaxed structure allowing the enrichment of the transcription factor specificity protein 1(SP1) at the *HSD11B2* promoter resulting in increased transcription of *HSD11B2*. This diagram is based on the work described in Refs. [45, 48, 70, 74]

phosphorylation, and increases in Sp1 and p300 along with the robust 11β-HSD2 expression [45, 48, 70]. Concomitantly, the enrichment of H3K27me3 is decreased while the H3K27ac is increased at the *HSD11B2* promoter during syncytialization [45, 48]. Consistent with these findings, a recent study demonstrated that inhibition of histone deacetylase can indeed promote 11β-HSD2 expression in JEG3 cells, a choriocarcinoma cell line [75].

Other signaling pathways involved in the regulation of 11β-HSD2 expression in the placenta

In addition to the above-described hCG-activated signaling pathways and transcription factor, a number of other hormones produced by the syncytialized trophoblasts, signaling pathways and transcription factors may also be involved in the regulation of *HSD11B2* expression in placental trophoblasts. For instance, in addition to hCG,

corticotropin-releasing hormone (CRH) is also produced in large quantities and activates the cAMP/PKA pathway during syncytialization. It has been shown that CRH indeed increases the abundance of 11β-HSD2 in primary human placental trophoblasts [76]. Of note, binding sites for potential transcription factors including nuclear factor 1 (NF1), Arnt1, Ah-Arnt, AP2, AP4 and Ik2 have been revealed in the methylated CpG sites in the *HSD11B2* promoter by in silico analyses [66]. Methylation of these CpG sites is shown to diminish the binding activities of not only Sp1 but also NF1 and Arnt [66]. Moreover, inhibition of the mitogen-activated protein kinases, ERK1/2, increases *HSD11B2* expression [77], while suppressing p38 decreases 11β-HSD2 activity [78]. Moreover, activation of both peroxisome proliferator-activated receptor delta (PPARδ) and the hedgehog signaling pathways are associated with increased *HSD11B2* expression [79, 80]. Emerging data also indicate that these signaling pathways appear to form a complex network in the regulation of *HSD11B2* expression during syncytialization. For example, the cAMP/PKA pathway activated by hCG has been shown to interact with the ERK1/2 and p38 pathways [81], while activation of the PPARγ pathway has been shown to be associated with Sp1 in the regulation of *HSD11B2* expression in placental trophoblasts [82]. However, the exact interactions among the complex repertoire of signaling pathways in the regulation of *HSD11B2* expression during syncytialization await further investigation.

Effects of adverse conditions on HSD11B2 expression in the placenta

The epigenetic modifications are very susceptible to environmental signals and can be altered at critical periods of development [83]. Accumulating evidence indicates that adverse conditions in pregnancy such as stress and nutritional restriction can result in intrauterine growth restriction which is possibly associated with the increased methylation levels of the CpG island in the promoter of *HSD11B2* gene. The increased methylation levels lead to decreased expression of *HSD11B2*, which can then compromise the placental glucocorticoid barrier, resulting in overexposure of the fetus to maternal glucocorticoids. Overexposure to glucocorticoids causes fetal growth retardation and can cause permanent changes in the expression pattern of the genes associated with the development of chronic diseases in later life.

Intrauterine growth restriction

Intrauterine growth restriction (IUGR) is a condition defined as a fetus being less than 10% of its estimated fetal weight for a particular gestational age. The causes may vary but most often involve nutrition restriction, hypoxia and stress. Epidemiological evidence indicates that IUGR is associated with an increased risk of hypertension, diabetes and obesity in later life, and overexposure of the fetus to maternal glucocorticoids has been identified as a potential underlying mechanism [8, 84, 85]. A considerable body of evidence has been building up in recent years for the correlation between reduced placental 11β-HSD2 expression and IUGR in human pregnancies [52, 54, 86, 87]. Catch-up growth is often seen after IUGR in the first year of extra-uterine life. Interestingly, Tzschoppe et al. showed that the expression of 11β-HSD2 in the placenta correlated not only positively with the birth weight but also inversely with the growth velocity in the first year of extra-uterine life of the IUGR baby [88], further indicating a crucial role of placental 11β-HSD2 in the protection from IUGR. The enhancement of methylation levels in the *HSD11B2* promoter is emerging as the major mechanism underlying the reduced 11β-HSD2 abundance in IUGR [89–92]. Marsit et al. reported that the extent of methylation of the *HSD11B2* promoter in the placenta was greatest in infants with the lowest birth weights [92]. Unfortunately, there is little information regarding the precise mechanisms underlying the methylation of the *HSD11B2* promoter. The mechanisms involved may differ depending on the causes of IUGR.

Stress

Different stressors can be encountered in pregnancy. Notably, both human and animal studies have shown that acute and chronic stress may have differential effects on the expression of 11β-HSD2 in the placenta [93–97]. While acute stress stimulates the expression of 11β-HSD2, chronic stress inhibits the expression of 11β-HSD2 in the placenta [93–97]. In agreement with these findings, acute treatment of cultured placental trophoblasts with glucocorticoids also increased the expression of *HSD11B2* [74, 98]. It is likely that the up-regulation of placental 11ß-HSD2 by acute stress may be an immediate protective measure adopted by the fetus against the sudden elevation of maternal glucocorticoids, while the inhibition of placental 11ß-HSD2 by chronic stress may be a strategy adopted by the fetus for its survival because chronic stress is a risk factor for preterm birth [99]. For this reason, the inhibition of placental 11ß-HSD2 by chronic stress enables more cortisol to pass into the fetal circulation thereby promoting the maturation of vital fetal organs for its survival in extra-uterine life. However, this survival strategy occurs at the expense of growth restriction and therefore increases the risk of chronic diseases in later life.

The mechanisms underpinning the differential regulation of placental 11ß-HSD2 expression by acute and chronic stress are not fully understood. However, studies have shown that the stimulation of placental 11ß-HSD2 expression by

glucocorticoids occurs at both transcriptional and post-transcriptional levels and is mediated by glucocorticoid receptors [74, 98]. At the transcriptional level, glucocorticoid-stimulated hCG production may account, at least in part, for the stimulation of *HSD11B2* expression by these hormones [74]. Accumulating evidence has demonstrated that chronic stress-induced reduction in placental 11ß-HSD2 expression may be associated with the hypermethylation of the *HSD11B2* gene promoter [91, 97, 100]. Although the detailed mechanisms underlying the hypermethylation of the *HSD11B2* gene promoter by chronic stress is not known, increased expression of the DNA methyltransferases may be involved in this process [97, 101]. Interestingly, chronic stress-induced hypermethylation of the *HSD11B2* gene promoter appears to be sexually dimorphic affecting mainly the female offspring [101–104].

Hypoxia

Maternal hypoxia is a common perturbation that can disrupt placental function and slow down fetal development, thus contributing to neonatal impairment. A study in mice showed that maternal hypoxia during mid- to late gestation not only changed the placental morphology but also reduced fetal birth weight with altered gene expressions including reduced MR and GR as well as *HSD11B2* expression in the placenta [105]. Human studies also demonstrated that the expression of *HSD11B2* was decreased when fetal asphyxia is present in the late phase of IUGR pregnancies [106].

In vitro studies using human placental trophoblasts also showed that when the cells were exposed to hypoxic conditions, both the induction of 11ß-HSD2 and syncytialization of trophoblasts were prevented [107, 108]. A further study revealed that hypoxia may induce an initially rapid down-regulation of 11ß-HSD2 protein synthesis at the translation level, and a later down-regulation of *HSD11B2* gene transcription [109]. However, it is not clear whether this transcriptional mechanism involves epigenetic modification of the *HSD11B2* gene.

Preeclampsia is characterized by shallow trophoblast invasion with subsequent hypoxemia. Studies in preeclampsia demonstrated no significant change in the methylation level in *HSD11B2* gene promoter [67, 110], despite the decrease of 11ß-HSD2 abundance seen in the placental tissue in this condition [53, 67, 82, 111]. These data suggest that hypoxia may affect placental *HSD11B2* expression through mechanisms other than modification through methylation.

Nutrition

The effect of nutritional restriction on birth weight and subsequent disease during adulthood is well demonstrated in studies of exposure to famine, most notably seen during the Dutch Hunger Winter [112, 113] as well as by studies in animals [114–116]. Overexposure to glucocorticoids during the critical window of fetal development is believed to be the critical mechanism underlying the early life programming of adult diseases in maternal nutritional restriction [117], suggesting that lack of nutrition can reduce the expression of *HSD11B2* in the placenta. Indeed, a number of studies in animals have demonstrated that maternal dietary intake restriction [118, 119] or protein content restriction [120–122] during early- to mid-gestation can result in decreased placental *HSD11B2* expression.

An in vitro study using BeWo cells, a trophoblast cell line, revealed that low levels of amino acids might decrease 11β-HSD2 abundance through leptin-activated JAK-STAT and MAPK signaling pathways [123]. However, an alternative mechanism for this phenomenon involves the possibility of modification of DNA methylation which remains a highly probable option in nutritional restriction because this process depends on the availability of methyl group donors and cofactors produced by methionine and folate metabolism or is provided by dietary-derived vitamins B6 and B12 [124]. However, limited data are available at present regarding the effect of dietary components on the methylation modification of the *HSD11B2* gene in the placenta. Of the few studies addressing this issue, investigation of the effect of dietary folic acid on the methylation level of the *HSD11B2* gene in the placenta is in favor of the methylation modification of *HSD11B2* gene in the placenta by dietary components [104]. In a similar experimental paradigm, folate supplementation during pregnancy was shown to be able to prevent much of the adverse programming effects of maternal protein restriction on the cardiovascular system in the offspring [125], suggesting that folate component in the diet may play a critical role in the modification of *HSD11B2* gene methylation in the placenta.

Summary and future perspectives

The placenta is equipped with a glucocorticoid barrier for the protection of the fetus from the adverse effects of excessive maternal glucocorticoids in almost all placental mammals including humans. This barrier is enforced by the expression of 11β-HSD2 in the syncytial layer of the placental villi, which converts biologically active cortisol into inactive cortisone. Impairment of this placental glucocorticoid barrier is associated with fetal IUGR and development of chronic diseases in later life. Ontogeny studies show that the expression of 11β-HSD2 is initiated at a very early stage after conception and increases with gestational age but declines around term. The promoter of *HSD11B2,* the gene encoding 11β-HSD2, has a highly GC-rich core. However,

the pattern of methylation marks on *HSD11B2* may have already been set up when the trophoblast identity of the blastocyst is committed. Instead, pregnancy hormones, such as the hCG-initiated signaling pathway, appear to be responsible for the upsurge of 11β-HSD2 expression during trophoblast syncytialization. By activating the cAMP/PKA pathway, hCG not only alters the modification of histones but also increases the expression of Sp1 which activates the transcription of *HSD11B2*.

Different causes of IUGR such as stress, hypoxia and nutritional restriction can be encountered in pregnancy. Available studies indicate that different causes of IUGR may attenuate *HSD11B2* expression in the placenta by differential mechanisms. While stress and nutritional restriction may reduce *HSD11B2* expression by increasing its methylation, hypoxia may decrease *HSD11B2* expression via an alternative mechanism rather than methylation. However, the detailed mechanisms underlying the attenuation of *HSD11B2* expression by adverse conditions have yet to be elucidated. We are unclear about how the methylation pattern of the *HSD11B2* promoter is changed by these adverse conditions and neither do we know the exact alternative pathways underpinning the reduction of *HSD11B2* expression by other conditions in the placenta. In addition, it still remains a puzzle when and where HSD11B2 expression is initiated in the blastocyst before the 3rd week of gestation. We believe that elucidation of these issues may help to develop strategies to provide a safer intrauterine environment for the development of fetus.

Acknowledgements This work was supported by National Natural Science Foundation of China (31671566 and 81330018), National Key R&D Program of China (2017YFC1001403) and National Key Basic Research Projects (2014CB943302). The authors would like to thank Dr. Dev Sooranna, Imperial College London, for editing the manuscript.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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