Death or survival – progesterone-dependent cell fate decisions in the human endometrial stroma

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Abstract

The human endometrium undergoes cyclical waves of proliferation, differentiation and apoptosis in response to the rise and fall in ovarian oestradiol and progesterone levels. These hormonal responses in endometrial cells must be tightly kept in check to safeguard tissue homeostasis throughout reproductive life. The discovery that differentiating endometrium highly expresses the tumour suppressor p53, the forkhead transcription factor FOXO1, and promyelocytic leukaemia zinc finger protein (PLZF) has provided new insights into the molecular basis of life and death decisions in response to sex steroid hormones.

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Introduction

The endometrium is the mucosa, characterised by glands and stroma, which lines the lumen of the uterus. During the reproductive phase, ovarian oestradiol induces the ordered growth of the endometrium whereas the postovulatory rise in progesterone levels controls differentiation of this tissue in preparation for an implanting embryo. In the absence of pregnancy, ovarian hormone levels fall and the endometrial epithelial cells regress. At least this is the scenario in most viviparous species. In humans, Old World monkeys, the elephant shrew and certain bats, the fall in progesterone levels does not induce regression of the endometrium but menstruation; a complex process characterised by shedding of the entire superficial endometrial layer with associated bleeding. Why some but not other species menstruate remains a major and yet unresolved conundrum in reproductive biology; not in the least because of the huge health burden associated with menstrual disorders. Many hypotheses have been advanced although only one is based on a distinct histological difference in the endometrial response to ovarian hormones between menstruating and non-menstruating species. Finn noted that progesterone triggers much more complex endometrial changes in menstruating species when compared with other mammals (Finn 1987, 1998). These changes not only involve the endometrial epithelial cells as in most mammals but also the cells, matrix and blood vessels of the stroma. This remodelling of the stromal compartment is termed ‘decidualisation’ and is characterised by massive influx of specialised uterine natural killer (uNK) cells, morphological and biochemical differentiation of endometrial stromal cells (ESCs) into decidual cells, and profound angiogenesis. Decidualisation occurs in all species where the implanting embryo breaches the endometrial surface epithelium and invades the maternal tissue (Brosens et al. 2002). The difference is, however, that decidualisation in humans and other menstruating species is no longer dependent on signals derived from the implanting blastocyst but is an autonomous maternally driven process (Dey et al. 2004).

The cyclical waves of proliferation, differentiation, shedding and regeneration of human endometrium, which occur on average 400 times during reproductive life, are unparalleled in any other tissue of the body. Within this cycle, decidualisation of the stromal compartment represents a tipping point after which the fate of the endometrium becomes irreversibly dependent upon progesterone signalling. In pregnancy, persistently elevated progesterone levels ensure survival and integrity of the decidua during the process of trophoblast invasion and placenta formation. In the absence of pregnancy, falling levels of progesterone trigger a cascade of events that results in proteolytic breakdown of the superficial endometrium, focal bleeding and cell death (Finn 1998, Critchley et al. 2001). In this review, we summarise the
molecular cues that underpin the decidual process and explore the role of three functionally related transcription factors, p53, FOXO1 and PLZF, in life and death decisions of human endometrial stromal cells.

Decidualisation of the endometrial stroma

The role of the decidual process in pregnancy and menstruation

Decidualisation is first apparent in the stromal cells surrounding the terminal spiral arteries of the superficial endometrial layer around day 23 of a 28-day cycle. It heralds the end of the limited period of endometrial receptivity (the ‘implantation window’) during which embryo attachment can take place. The elongated spindle-like stromal cells transform into cobblestone-like enlarged decidual cells with multiple club-shaped projections arising from their cell surface (Wynn 1974). In pregnancy, the endometrium is under the continued support of steroid hormones as well as blastocyst-derived signals such as human chorionic gonadotrophin (hCG). This provides the hormonal milieu for full decidualisation of the entire endometrium. The decidua forms a dense cellular matrix that allows coordinated trophoblast invasion while simultaneously protecting the conceptus from maternal and environmental insults (Kliman 2000, Red-Horse et al. 2004).

The distinct morphological appearance of decidualising stromal cells is underpinned by profound biochemical changes. Microarray studies have demonstrated that decidualisation involves sequential reprogramming of functionally related families of genes involved in extracellular matrix organisation, cell adhesion, cytoskeletal organisation, signal transduction, metabolism, differentiation and apoptosis (Giudice 2004). Consequently, upon biochemical reprogramming, the ESCs acquire many new functions that critically govern successful trophoblast invasion and placenta formation. For instance, differentiating ESCs secrete a variety of factors, such as macrophage inflammatory protein-1β, interleukin (IL)-11, IL-15 and prolactin (PRL), which provide the chemotactic, proliferative and differentiating signals for specialised immune cells (Dimitriadi et al. 2005). Most abundant are uNK cells, which constitute 50–90% of the resident lymphocytes in early gestation. These innate immune cells are a rich source of cytokines and angiogenic growth factors. They are not only involved in conferring immunotolerance towards paternal antigens expressed on the invading trophoblast but also play a role in the remodelling of the spiral arteries prior to endovascular trophoblast invasion (Dosiou & Giudice 2005, Leonard et al. 2006). The decidua also expresses factors that are implicated in the suppression of T cell-dependent immune responses to fetal alloantigens such as the tryptophan-catabolising enzyme indoleamine 2,3-dioxygenase (IDO), tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FasL) (Popovici et al. 2000, Kayisi et al. 2003, Kudo et al. 2004). Remodelling of the extracellular matrix, characterised by the production of laminin, type IV collagen, fibronectin, and heparan sulphate proteoglycan, and secretion of growth factors and binding proteins such as insulin-like growth factor binding protein-1 (IGFBP-1) critically regulate coordinated trophoblast invasion and differentiation (Fazleabas et al. 2004, Kayisi et al. 2005). Implantation and formation of the placenta are inflammatory processes, and differentiating ESCs express enzymes such as mitochondrial manganese superoxide dismutase (MnSOD) that are involved in oxidative stress defences (Sugino et al. 1996).

Furthermore, decidualising stromal cells surrounding the spiral arteries highly express tissue factor (TF, the initiator of the extrinsic coagulation pathway) and plasminogen activator inhibitor-1 (PAI-1, a fibrinolysis inhibitor), emphasising their primary role in maintaining vascular stability prior to menstruation and during endovascular trophoblast invasion (Schatz et al. 2003).

In the absence of pregnancy, falling sex hormone levels induce a switch in the secretory repertoire of differentiated ESCs that triggers a sequence of events leading to menstrual shedding of the superficial endometrial layer. Perhaps the first histological sign of impending endometrial breakdown is uNK cell apoptosis. As uNK cells do not express progesterone receptors (PR), cell death is likely a consequence of paracrine signals derived from PR-positive stromal cells (King 2000). Upon progesterone withdrawal, differentiated stromal cells also produce the chemokines and inflammatory mediators that trigger local infiltration of the endometrium by macrophages and other inflammatory cells. Approximately two days before the onset of menstruation, endometrial epithelial cells show a high degree of apoptosis, a low proliferative index and loss of PR expression. In contrast, stromal cell apoptosis is a later event, which is less pronounced and coincides with increased proliferation rate, persistent PR expression and decreased levels of the anti-apoptotic Bcl-2 protein. These characteristics indicate a high cell turnover in the stromal compartment during menstruation in preparation for the subsequent tissue regeneration (Dahmoun et al. 1999). Conceivably, even limited apoptosis of decidualised cells surrounding the terminal spiral arteries may trigger focal interstitial haemorrhage. Ultimately, the shedding of endometrial tissue requires local and controlled degradation of the extracellular matrix, which in turn is dependent upon the balance between matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). MMPs form a large family of zinc-dependent enzymes with considerable structural homology but distinct substrate specificities (Dong et al. 2002). Although all cellular components in the superficial
Hormonal signals for decidualisation

Decidualisation is beautifully reciprocated in cultured ESCs upon addition of the appropriate hormonal stimuli. Many protocols for in vitro decidualisation have been developed over the last 25 years. Three major points can be deduced from these studies. First, treatment with progesterone for eight or more days, alone or in combination with oestradiol, can induce expression of decidual markers such as PRL or IGFBP-1 in primary cultures, albeit at modest levels. Second, ligands that trigger a persistent increase in intracellular cyclic AMP (cAMP) levels much more rapidly induce expression of decidual marker genes, although the levels are not maintained in long-term cultures. Finally, full decidualisation and sustained expression of the differentiated phenotype requires both elevated cAMP levels and progesterone (Gellersen & Brosens 2003). These culture conditions reflect the in vivo situation. The endometrial stroma in the secretory phase of the cycle is not only exposed to progesterone but also to a number of factors which bind to G-protein coupled receptors (GPCRs) present in stromal cells, resulting in activation of adenylate cyclase, formation of the second messenger cAMP and activation of protein kinase A (PKA). Such ligands include prostaglandin E₂, corticotrophin releasing hormone, relaxin and perhaps the circulating pituitary gonadotrophins (Gellersen & Brosens 2003). In pregnancy, trophoblast-derived hCG may further act on gonadotrophin receptors expressed on differentiating endometrial cells. While cAMP can also act in a PKA-independent fashion by binding to the Rap guanine exchange factors Epac (exchange protein activated by cAMP) 1 and 2 (de Rooij et al. 1998, Kawasaki et al. 1998), this pathway does not appear to play a significant role in cAMP-induced decidualisation (Gellersen & Brosens 2003).

In recent years, the regulation of major decidual marker genes, such as PRL, IGFBP-1, and TF, has been widely used as a paradigm for the dissection of the cross-talk between cAMP and progesterone signalling in ESCs. Progesterone actions are mediated by binding and activation of PR, a member of the nuclear receptor superfamily of ligand-dependent transcription factors. Notably, none of the major decidual genes appears to be under direct transcriptional control of activated PR, in keeping with the inability of progesterone to induce ESC differentiation in short-term cultures. This may be attributable to the relative level of expression of two PR isoforms, PR-A and PR-B, which arise from differential promoter usage in a single gene. PR-A lacks the 164 N-terminal amino acids of PR-B, is transcriptionally less active and can transrepress PR-B on palindromic progesterone-responsive elements (Li & O’Malley 2003). It is however the dominant isoform in human decidualising ESC in vivo and in vitro, and in mice, selective deletion of PR-A leads to a defective decidual response to the implanting blastocyst (Brosens et al. 1999, Mote et al. 1999, Mulac-Jericevic & Conneely 2004). In human ESC, emerging evidence suggests a major role for the activated PR, specifically the PR-A isoform, as a scaffold for the recruitment of transcription factors activated, directly or indirectly, in response to cAMP signalling (Gellersen & Brosens 2003). Direct physical interaction has indeed been demonstrated between PR and signal transducer and activator of transcription 5 (STAT5), CCAAT enhancer-binding protein β (C/EBPβ), or forkhead box O (FOXO1) (Owen et al. 1998, Richer et al. 1998, Christian et al. 2002a, Kim et al. 2005). By hijacking these transcription factors, the activated PR acquires control of the diverse gene families involved in decidualisation. More recently, it has become apparent that the cAMP and progesterone signalling pathways also differentially control the expression and activity of a limited number of transcription factors involved in cell fate decisions.

Transcription factors in endometrial stroma with implications in cell fate decisions

PLZF – a progesterone-inducible antiproliferative factor that confers resistance to apoptosis

Structure and function of PLZF

The promyelocytic leukaemia zinc finger protein (PLZF) was first identified in acute promyelocytic leukaemia where a reciprocal chromosomal translocation results in expression of fusion proteins between PLZF and retinoic acid receptor α. The wild-type PLZF belongs to the large family of POZ domain and Krüppel zinc finger (POK) proteins that contain a conserved N-terminal BTB/POZ (bric-a-brac/tramtrack/broad complex, poxvirus and zinc finger) repressor domain and a C-terminal DNA-binding region composed of zinc fingers of the Krüppel type. PLZF has a second repressor domain that interacts with the Eight-Twenty One (ETO) protein. Both PLZF and ETO recruit proteins such as SMRT (silencing mediator for retinoid and thyroid-hormone receptors), N-CoR (nuclear receptor co-repressor), Sin3 and HDACI (histone deacetylase 1) to form a multiprotein transrepressor complex (Melnick et al. 2000, Costoya & Pandolfi 2001). In development, PLZF is crucial for limb and axial skeletal patterning, forebrain organisation and hindbrain segmentation (Avantaggiato et al. 1995, Cook et al. 1995,
Barna et al. (2000). With regard to reproductive functions, gene deletion studies in mice revealed an essential role for this factor in maintaining spermatogonial stem cells in adults. In addition, PLZF was identified as an androgen-responsive gene in the prostate (Buasas et al. 2004, Costoya et al. 2004, Jiang & Wang 2004). PLZF can confer resistance to apoptosis, partly by transcriptional inhibition of the gene encoding the pro-apoptotic BID protein, a member of the Bcl-2 family (Parrado et al. 2004). Further, PLZF exerts an anti-proliferative effect through suppression of cyclin A transcription (Yeyati et al. 1999).

**PLZF in the endometrial stroma**

We identified PLZF as an early progesterone-inducible product in cultured human ESCs. Within 2 h of progesterone treatment, PLZF mRNA levels are massively up-regulated while treatment with a cAMP analogue has no effect on its expression. In vivo, PLZF accumulates in the nuclei of stromal cells in the mid-late secretory endometrium (Fahnenstich et al. 2003). Studies in human fibrosarcoma cells indicated that the antiproliferative action of PLZF is reversed by HB–EGF-C, the C-terminal soluble fragment that results from proteolytic cleavage of the membrane-anchored precursor proHB–EGF (heparin-binding epidermal growth factor-like growth factor). HB–EGF-C translocates to the nucleus, interacts with PLZF and triggers its nuclear export, thus reversing cell cycle inhibition (Nanba et al. 2003). In human ESCs, HB–EGF expression is induced by cAMP, enhances the expression of decidual marker proteins, and confers resistance to apoptosis induced by pro-inflammatory cytokines (Chobotova et al. 2005). While the N-terminal cleavage product of proHB–EGF, the mature HB–EGF, is a growth-promoting ligand of the EGF receptor, the role of the by-product HB–EGF-C in ESCs has not been assessed. It is nevertheless tempting to speculate that an altered balance between progesterone-induced antiproliferative and antiapoptotic PLZF and cAMP-induced HB–EGF-C participates in endometrial stromal cell fate decision towards the end of the menstrual cycle.

**p53 – a cAMP-inducible proapoptotic, antiproliferative protein with functions beyond tumour suppression**

**Structure, regulation and tumour suppressor function of p53**

Structurally, p53 is a transcription factor with an N-terminal transactivation domain, a core DNA-binding region and a C-terminal tetramerisation domain. Among the target genes activated by p53 are those encoding the pro-apoptotic Bax, NOXA and PUMA members of the Bcl-2 family, the cell cycle inhibitor p21<sup>WAF1</sup>, growth arrest and DNA damage-inducible gene 45 (GADD45), and mouse double minute-2 (Mdm2) (Levine 1997). Induction of Mdm2 establishes an important negative feedback loop as it binds to p53 at its N-terminus, functions as an E3-ubiquitin ligase, and targets p53 for proteasomal degradation. Transcriptionally inactive p53 mutants, which are found in approximately 50% of all human tumours, fail to induce Mdm2 and consequently p53 accumulates in tumour cells (Lane & Hall 1997, Vogelstein et al. 2000). This phenomenon led originally to the misconception that p53 is an oncogene. A further player in the p53/Mdm2 regulatory loop is the ARF protein (p14<sup>ARF</sup> in human, p19<sup>ARF</sup> in the mouse), the product of an alternative transcript from the CDKN2A tumour suppressor locus. It binds to Mdm2, inhibits its activity, and thus allows p53 to escape from Mdm2-mediated degradation (Stott et al. 1998, Oren et al. 2002). Significantly, the CDKN2A locus is frequently deleted or silenced in different tumours (Sharpless & Chin 2003).

While the TP53 gene is constitutively transcribed, the level of p53 protein is kept extremely low under physiological conditions through rapid proteasomal degradation of newly synthesised protein. Only in response to genotoxic insults, such as exposure to UV light, irradiation, oncogene activation, hypoxia or chemotherapeutic agents, p53 is stabilised, accumulates rapidly, and becomes transcriptionally active. It then functions, relating to its role as a tumour suppressor, to induce cell cycle arrest or apoptotic cell death. In addition to cell cycle arrest in G1, p53 can also arrest cells in G2 and inhibit entry into mitosis. This occurs partly through induction of three p53 target genes (GADD45, 14–3–3 and p21<sup>WAF1</sup>) that simultaneously inhibit transcription of the Cdc2 cyclin-dependent kinase (Taylor & Stark 2001). Cell cycle arrest may be permanent or transient, allowing repair of damaged DNA before resumption of replication. Apoptosis will irreversibly remove damaged cells from the organism. Both mechanisms serve to prevent the emergence of cell populations harbouring oncogenic mutations (Ryan et al. 2001).

Subcellular localisation, activity and stability of p53 are interdependent and subject to complex regulation by post translational modifications including phosphorylation, acetylation and sumoylation (Ryan et al. 2001). In addition to direct transcriptional activation of target genes, p53 exerts many of its functions through protein/protein interactions. In fact, gene expression profiling has revealed that many responsive genes are actually repressed by p53 through both direct and indirect mechanisms (Mirza et al. 2003, Kho et al. 2004).

**p53 in the endometrial stroma**

In the course of cAMP-induced decidualisation, high levels of nuclear p53 protein accumulate in cultured
ESC, and intense nuclear p53 immunoreactivity is observed in endometrial stromal cells during the secretory phase of the cycle (Pohnke et al. 2004). There are several interesting aspects to p53 expression in human endometrial stroma. First, the kinetics of its induction in response to cAMP signalling are not typical of a stress response. Expression of p53 is a delayed response, requiring between 2 to 4 days of cAMP stimulation, and coincides with the appearance of morphological and biochemical signs of decidualisation. Secondly, the accumulation of p53 protein is not due to enhanced transcription of the TP53 gene but a consequence of protein stabilisation. Finally, progesterone does not induce or enhance p53 expression or alter its DNA-binding activity (Pohnke et al. 2004).

What function might p53 serve in the decidualising endometrium? It is unlikely that p53 induces extensive apoptosis in differentiating cells. While cAMP signalling modestly increases the apoptotic fraction in culture, this only affects approximately 12–15% of cells, whereas p53 accumulation occurs in virtually all cells (J J Brosens & E W-E Lam, unpublished observations). Instead, there appears to be a close link between p53 levels and the differentiation status of ESCs. For instance, withdrawal of the differentiation stimulus from decidualised cells results in reversal of the differentiated to the undifferentiated proliferative phenotype along with disappearance of p53 protein levels. It is possible that increased p53 levels in decidualised stromal cells during the secretory phase of the cycle constitute a pool of transcriptionally inert proteins that are activated upon withdrawal of survival signals prior to menstruation. In a non-activated state, p53 could still exert repressor function by protein/protein interactions. We observed direct physical association and transrepression between p53 and the leucine zipper transcription factor C/EBPδ which is an important mediator of the cAMP signal in decidualising ESC (Christian et al. 2002a,b, Schneider-Merck et al. 2006). Transcriptionally activated p53 in turn might be involved in regulation of MMP expression. A perfect p53 binding site has been identified in the MMP-2 promoter, contiguous with binding motifs for activating protein-2 (AP2) and YB-1. Interaction between these transcription factors yields ternary complexes that lead to synergistic activation of MMP-2 transcription (Bian & Sun 1997, Mertens et al. 2002). It is thus conceivable that p53 in decidualised ESC participates in MMP-2 expression, a prerequisite for endometrial breakdown and subsequent tissue regeneration. Importantly, MMP-2 expression is up-regulated in decidualised ESC upon progesterone withdrawal (Irwin et al. 1996), indicating the release from a suppressive mechanism.

Accumulation of wild-type p53 in the absence of stress is an unusual phenomenon in most cells but not in female reproductive tissues. In the ovary, p53 appears to act as a pro-apoptotic mediator involved in maintaining tissue homeostasis during the cyclical process of follicle selection (Makrigiannakis et al. 2000). A different role is suggested for p53 in normal mammary tissue. In the quiescent mammary gland, p53 localises to the cytoplasm of the ductal epithelium and appears refractory to activation in response to ionising radiation. However, upon 6 h of treatment with hCG, p53 is recruited to the nucleus and can be functionally activated by irradiation to induce target gene transcription (Kupperwasser et al. 2000). In the rat, elevated oestrogen and progesterone levels during pregnancy also induce sustained nuclear accumulation of p53 capable of responding to carcinogenic challenges. This ability of nuclear p53 to survey and maintain genome integrity in breast tissue is thought to account for the protective effect of pregnancy against the incidence of breast cancer. In the murine mammary epithelium, high nuclear levels of p53 are maintained throughout pregnancy, weaning and involution (Sivaraman et al. 2001). The elevated p53 levels may partly be attributable to a progesterone-dependent increase in the levels of tumour suppressor p19ARF during pregnancy, lactation and involution. The role of p19ARF could be to sustain a normal proliferation rate of the mammary epithelium in pregnancy while inducing apoptosis in a p53-dependent fashion upon weaning and involution (Yi et al. 2004). The decidua also expands phenomenally during pregnancy to accommodate the growing fetus. Conceivably, decidual p53 may therefore also serve as a ‘guardian of the genome’ during gestation. Importantly, both activated and non-activated p53 can serve complementary roles in the maintenance of genome integrity (Albrechtsen et al. 1999).

FOXO1 – a cAMP-inducible, progesterone-regulated switch at the crossroads of apoptosis and survival

Structure and function of FOXO1

The FOXO (forkhead box O) proteins constitute a subclass of the winged helix of Forkhead box (FOX) class of transcription factors which are helix-turn-helix DNA-binding proteins with a butterfly-like appearance. FOXO transcription factors are critical mediators in cell fate decisions in response to growth factor, hormonal and environmental cues (Accili & Arden 2004). Although structurally unrelated, they share striking functional homologies with p53. Both are involved in the control of cell cycle arrest and the induction of apoptosis. This is, at least in part, attributable to the fact that they share common target genes such as GADD45, p21WAF1 and FasL (You & Mak 2005).

The transcriptional activity of FOXO proteins is critically regulated by their subcellular localisation. Growth factor signalling through the phosphatidylinositol 3-kinase (PI3K) pathway leads to phosphorylation of the serine/threonine kinase Akt (also termed protein
kinase B) that in turn phosphorylates downstream target proteins, including the FOXO transcription factors. Akt-dependent phosphorylation of nuclear FOXO generates binding sites for the 14–3–3 chaperone protein which releases FOXO factors from their target DNA and causes translocation to the cytoplasm. Growth factor-dependent nuclear exclusion and inactivation of FOXO thus leads to reduced expression of proapoptotic and antiproliferative target genes and promotes cell survival (Brunet et al. 1999, Burgering & Kops 2002).

**FOXO1 in the endometrial stroma**

Of the three human FOXO proteins (FOXO1, FOXO3a, and FOXO4), FOXO1 is markedly induced upon decidualisation both in vivo and in vitro, and is involved in regulating the expression of decidual marker genes, such as PRL and IGFBP-1. The promoters of both genes are activated by multimeric transcription factor complexes containing FOXO1 which assemble in response to an interplay of cAMP- and progesterone-dependent signals (Christian et al. 2002b, Gellersen & Brosens 2003, Kim et al. 2003, 2005). Furthermore, the expression and activity of FOXO1 itself is subject to intricate control mechanisms involving both the PKA pathway and the ligand-activated nuclear PR. Within three days of cAMP treatment, cultured ESC up-regulate FOXO1 mRNA and protein, and this response is markedly enhanced by progestin although treatment with progestin alone does not induce FOXO1 expression (Labied et al. 2006). A known FOXO target gene is BIM (BCL2 L11) which encodes the proapoptotic Bcl-2 homology 3 domain-only protein Bim (Dijkers et al. 2000, Sunters et al. 2003). Bim expression increases in

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**Figure 1** Model for progesterone-dependent regulation of FOXO1 transcriptional activity in ESCs. Left panel: In the late luteal phase of the cycle, intracellular cAMP is elevated and synergises with progesterone (P4) to induce FOXO1 expression. P4, however, causes nuclear exclusion of a large portion of the FOXO1 protein pool which forms an inactivated standby reservoir. A small fraction of active FOXO1 remains in the nucleus and is recruited to transcription factor complexes assembled around the ligand-activated nuclear PR. Such transcriptosomes induce the expression of differentiation-specific decidual genes like PRL and IGFBP-1 and may include other transcription factors such as C/EBPβ, STAT5 or HOXA10 (Gellersen & Brosens 2003, Kim et al. 2003, 2005). Right panel: Upon withdrawal of progesterone prior to endometrial breakdown, the cytoplasmic FOXO1 fraction rapidly re-enters the nucleus. Here, the abundant active FOXO1 binds to its direct target genes, thereby inducing cell cycle arrest or apoptosis. The size of the red octagon symbolises the relative amount of active vs inactive FOXO1 protein.
the endometrium prior to menstruation and in culture upon activation of the cAMP pathway in a FOXO1-dependent manner. However, despite the fact that FOXO1 levels are even higher in cells treated with a combination of cAMP and progestin, Bim expression is inhibited under these conditions (Labied et al. 2006). The solution to this paradox lies in the fact that cAMP causes nuclear accumulation of FOXO1 while added progestin induces nuclear exit and thus inactivation of a large fraction of the total FOXO1 protein pool. Withdrawal of progestin in turn results in rapid nuclear re-accumulation of FOXO1, increased expression of Bim and increased cell death (Labied et al. 2006). Another FOXO target gene, Fas ligand (FASLG), has also been implicated in regulating ESC apoptosis (Chatzaki et al. 2001, Kayisli et al. 2003) and is likely subject to similar regulatory mechanisms as described for BIM.

These findings suggest a critical role for FOXO1 in mediating the proapoptotic pathway initiated by progesterone withdrawal at the end of the menstrual cycle (Fig.1). This is underpinned by the observation that silencing of FOXO1 expression in differentiating ESC completely abrogates apoptosis induced by progestin withdrawal, suggesting that progesterone serves as a survival factor in decidualised ESCs through partial cytoplasmic retention and inactivation of FOXO1 (Labied et al. 2006). Interestingly, hormone-dependent nuclear exclusion of FOXO1 has also emerged as a survival mechanism in ovarian granulosa cells. Here, follicle stimulating hormone (FSH) provides the survival signal to prevent atresia in the antral follicles by promoting phosphorylation, cytoplasmic translocation and inactivation of FOXO1 in a PI3K-dependent fashion (Cunningham et al. 2003).

**Progestosterone – tipping the balance**

The above considerations on three structurally distinct, but functionally related transcription factors in the luteal phase endometrial stroma highlight a central role for progesterone as the master switch at the crossroads of death and survival. A speculative model for the progesterone-dependent cell-fate decision in decidualised ESCs is shown in Figure 2. In the luteal phase of the menstrual cycle, p53 and FOXO1 have accumulated under the influence of the activated PKA pathway, but progesterone-signalling has led to inactivation of a large portion of FOXO1 while PLZF expression has been upregulated. Under these conditions, the small active pool of FOXO1 preferentially induces differentiation-specific decidual genes, and the pro-survival function of PLZF outweighs the proapoptotic potential of p53. Upon progesterone withdrawal, however, PLZF expression is no longer supported, while FOXO1 is rapidly and massively re-activated from the cytoplasmic standby pool and cooperates with p53 in the initiation of apoptosis.

**Perspective**

The sheer extent of tissue remodelling in the human endometrium prior to and during pregnancy is unsurpassed anywhere in the body. To date, little attention has been paid to the molecular safeguards that ensure endometrial homeostasis, which is perplexing as
survival of the species is dependent upon these mechanisms. Coordinated endometrial remodelling requires precise hormonal regulation of transcription factors that control opposing gene programmes involved in cell fate decisions. Perturbation of this system is implicated in the molecular pathogenesis of common reproductive disorders, including endometriosis and endometrial cancer. Endometriosis is a debilitating condition that is characterised by ectopic growth of endometrial tissue and affects 5–10% of women of reproductive age. FOXO1 expression is lower in the endometrium of patients with endometriosis, and non-random somatic p53 locus alterations have been linked to late or severe-stage endometriosis (Shazand et al. 2004, Gylfason et al. 2005). Several studies have reported that apoptosis is markedly reduced in premenstrual endometrium of endometriosis patients as well as in the endometriotic lesions (Gebel et al. 1998, Dmowski et al. 2001), raising the possibility that dysregulation of FOXO1 expression contributes to implantation, survival and growth of ectopic endometrium. Endometrial cancer, on the other hand, is the commonest malignancy of the female reproductive tract and its incidence is increasing. In addition to lower FOXO1 expression, the majority of oestrogen-related type I (endometrioid) endometrial cancers are characterised by loss or mutation of the tumour-suppressor gene PTEN, leading to enhanced activity of the PI3K/Akt signalling pathway, and further inactivation of FOXO proteins (Kong et al. 1997, Risinger et al. 1998). Conversely, dominant-negative TP53 mutations are often found in advanced and histological subtypes of endometrial cancer and are a strong predictor of survival (Sakuragi et al. 2005). These clinico–pathological observations should accelerate research into the mechanisms that govern life and death decisions in the human endometrium.

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References


Cunningham MA, Zhu Q, Unter man TG & Hammond JM 2003 Follicle-stimulating hormone promotes nuclear exclusion of the forkhead transcription factor FoxO1 via phosphatidylinositol 3-kinase in proline granulosa cells. Endocrinology 144 5585–5594.
immunotolerance and endometrial apoptosis by regulating Fas-Fas ligand system. Journal of Immunology 171 2305–2313.


Sivaraman L, Connelly OM, Medina D & O'Malley BW. 2001 Five is a potential mediator of pregnancy and hormone-induced resistance to mammary carcinogenesis. PNAS 98 12379–12384.


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