Decidualization in Mouse and Man

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Abstract

Blastocyst implantation requires the best communication between the mother's uterus and a competent embryo. Even before the embryo enter the uterus, the endometrial tissue undergoes the process of decidualization to support the anchoring of the blastocyst and provides the blastocyst with nutrition until the fully functional placenta is formed. In this review, I summarize all the molecular and morphological events necessary for the decidualization in mice and human.

Reviews

Decidualization in mice is first initiated in the antimesometrial chamber where the blastocyst implants. The decidual process is characterized by fibroblast-like stromal cell proliferation then differentiation into specialized polyploid decidual cells. At the beginning of decidualization, only the stromal cells surrounding the implanting blastocyst proliferate and differentiate [1-3]. In mice, the stromal cells close to the implanting blastocyst cease to proliferate and differentiate; initiating primary decidual zone (PDZ) formation beginning on Day 4.5, and this PDZ is fully established by Day 5.5. This PDZ contains a high level of intercellular tight, gap, and adherent junctions and is avascular, thus, it provides a permeability barrier to extracellular macromolecules and cells and is believed to help protect the developing conceptus [4, 5]. The stromal cells outside the PDZ continue to proliferate, forming the secondary decidual zone (SDZ) [6, 7]. When decidualization continues toward the myometrium in the antimesometrial region forming the secondary decidual zone and in the mesometrial region forming the mesometrial decidua, the PDZ disappears. By Day 7.5, the conceptus is surrounded by cells of the antimesometrial and mesometrial decidual tissues that are highly vascular. At this time,

no permeability barrier exists, and maternal cells plus macromolecules may come into contact with cells of the conceptus. Therefore, the PDZ provides only a transient permeability barrier between the mother and conceptus [8].

Under normal condition, the decidual stimulus is a blastocyst. However, a similar process (deciduoma) can be experimentally induced in the pseudopregnant or hormonally prepared ovariectomized rodent by uterus intraluminal infusion of various agents like oil [1, 7]. In human, decidual reaction can happen in the late secretary phase of the menstrual cycle and terminate in menstruation if pregnancy does not happen. Thus, in human, decidualization begins without an "implantation stimulus".

Decidual tissues provide the nutritious environment for developing embryo before the establishment of the functional placenta. As mentioned above, the differentiation of uterus to support implantation is primary directed by progesterone [9, 10]. There is evidence that progesterone is important for enhancing and maintaining the decidual phenotype. It acts as a platform to integrate a numerous transcription factors which are largely unknown [11]. The following discussion will be dedicated to present the morphological and biological characteristics of the decidual endometrial stromal cells (ESCs) then focus on the signal and transcription factor networks that govern this differentiation process.

Morphological differentiation: Endometrial stromal cells are differentiated mesenchymal cells which have an elongated fibroblastic appearance in the proliferative phase of the menstrual cycle. Decidualization of the ESCs initiates in the cells that surround the terminal portion of the spiral arteries of the superficial endometrial layer during the mid-secretory phase. If menstruation occurs, decidualization does not go

beyond this. In mouse, the presence of the blastocyst or a physical implantation stimulus and endometrial epithelia are both essential for ESC decidualization to begin [12]. It is important that the onset of decidualization heralds the end of the limited period of uterine receptivity (Day 6.5 to Day 9.5 after ovulation in man). Ultrastructurally, in human the decidual cells are characterized by cell enlargement, rounding of the nucleus, with an increase in the number and complexity of nucleoli, expansion of the secretary apparatus with dilation of the rough endoplasmic reticulum, as well as cytoplasmic accumulation of glycogen and lipid droplets [13]. In vitro, the decidual phenotype can be acquired by culture the undifferentiated cells with ovarian hormones with cAMP or PGE2 [14], making it available in vitro model to study decidualization.

Biochemical characteristics: In human, major secretary product of decidual cells, such as prolactin (PRL) and insulin-like growth factor binding protein 1 (IGFBP1), have traditionally been used as markers of decidualization. Decidual prolactin is a member of the growth hormone/ placental lactogen/ prolactin family. Prolactin sequence of decidual prolactin cDNA is identical to pituitary prolactin sequence, but its expression is controlled by an alternative promoter [15]. Decidual PRL, as well as IGFBP1, seems to be important for fetal growth and development [16]. Other putative roles of decidual prolactin include an osmoregulatory function in the amniotic fluid, regulation of pulmonary surfactant synthesis in the fetus and maintenance of T-cell immuno-competence [16]. Recently, microarray-based genome-wide expression profiling has identified hundreds of genes that are either up or down-regulated during menstrual cycle in human [17-19]. These studies confirmed that the decidual process involves in sequential reprogramming of functionally related families of genes involved in cell

extracellular organization, cell adhesion, cytoskeleton organization, metabolism, signal transduction, differentiation and apoptosis. Upon biochemical reprogramming, the decidual cells may acquire new functions that govern the trophoblast invasion and placental formation. For example, decidual stromal cells surrounding the spiral arteries express tissue factor (TF- an initiator of the extrinsic coagulation pathway) and plasminogen activator inhibitor 1 (PAI1-a fibrinolysis inhibitor) indicating that they play an important role in maintaining the vascular stability prior to menstruation and during endovascular trophoblast invasion [20]. Differentiating ESCs secrete a variety factors such as interleukin (IL)-11, IL-6 and PRL, which are thought to provide chemotactic, proliferative and differentiating signal for uNK cells [21, 22]. However, decidual cells also expresses the tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO), tumor necrosis factor (TNF)-related apoptosis (TRAIL) and Fas ligand (FasL) which are implicated in suppression of T cell response to fetal alloantigens [23, 24]. Remodeling of the extracellular matrix (such as Laminin B1-LAMB1, tissue metalloproteinase inhibitory 3-TIMP3, collagen type IV-CLIV) and growth factors (Such as transforming growth factor beta-TGFβ) and binding proteins (such as IGFBP1) are thought to critically regulate trophoblast invasion and differentiation [25, 26]. In mouse, the equivalent decidual makers are alkaline phosphatase (*Akp2*), prolactin family 8-subfamily a-member 2 (*Prl8a2*), and *Runx1* [27-30].

Matrix remodeling and angiogenesis during decidualization: Endometrial tissue remodeling and angiogenesis are two hallmarks in implantation and decidualization. Hormonal milieu during the reproductive cycle and pregnancy results in extensive remodeling in the uterine tissue [31]. Various basement membrane components, such as

type IV collagen, laminin, fibronectin, and proteoglycans, in the human uterus undergo changes throughout menstrual cycle and pregnancy [31]. Likewise, extracellular matrix (ECM) components undergo remodeling during decidualization in mouse [32]. Matrix metalloproteinases (MMP) and tissue inhibitors of MMP (TIMP) are thought to be the major players for matrix remodeling in implantation and decidualization [31-33]. Although the mechanism regulating the expression of MMPs and TIMPs is not clear, there is evidence that the balance between a select set of MMPs and TIMPs is important for implantation.

Angiogenesis is the process by which new blood vessels develop from pre-existing vessels. This process is happened under normal physiological condition in the uterus and ovary of the adult during the menstrual cycle and pregnancy [34]. The increased vascular permeability and angiogenesis are critical for a successful implantation and decidualization. There is a number of studies reported indirect evidence of the potential roles of estrogen and progesterone in angiogenesis in various species [34-37]. The vascular endothelial growth factors (VEGFs) and its receptors (VEGFR1, VEGFR2) are known to regulate vascular permeability and angiogenesis [38, 39]. VEGF, originally discovered as a vascular permeability factor, is also a potent endothelial mitogen and a key regulator for vascular genesis and angiogenesis [38, 39]. Targeted disruption a single VEGF allele resulted in abnormal blood vessel development and embryo lethality in mice [40, 41]. In mouse, the expression of VEGF and its receptor as a whole are regulated by steroid hormone. For example, estrogen induced the vascular permeability and *Vegf* expression during pregnancy [38]. Further investigation reported that estrogen regulate *Vegf* transcriptionally via ER receptor, and the *Vegf* gene contains

estrogen consensus element *EREs* [42]. Progesterone also up-regulates *Vegf* expression but with a slower rate compared to estrogen [42]. Because estrogen rapidly induces the vascular permeability and *Vegf* expression, and because vascular permeability is pre-requisite for angiogenesis, people believed that estrogen is a potent stimulator of uterine angiogenesis during normal reproduction process *in vivo*. However, recent evidence showed that estrogen promotes vascular permeability and profoundly inhibits angiogenesis whereas progesterone stimulates angiogenesis with little effect of uterine permeability. These effects of E2 and P4 in uterus are mediated by differential spatiotemporal expression of pro-angiogenic factors [43]

Cell cycle regulation in decidualization: The mechanism by which the cell cycle events are governed in decidualization is poorly understood. The cell cycle is tightly regulated at two checkpoints at the G1-S and G2-M phases. Normal operation of these phases involves a complex interplay of cyclins, cyclin-dependent kinases (cdks) as well as cyclin-dependent kinase inhibitors (CKIs) [44]. In the human uterus, various cyclins (A, B1, D1, and E), cyclin-dependent kinases (cdk1, cdk2, cdk4) and CKI (p27) are regulated during normal menstrual cycle or hormone treatment [45, 46]. Those molecules primarily express in the epithelia and stromal cell in the uterus during the proliferative phase suggest their roles in rhythmic proliferation of these cells [46]. On the other hand, during the secretary phase or after progesterone administration, p27 expression correlates with progesterone induced growth suppression in endometrial gland and stromal basalis [47]. In rodents, the expression of D and E cyclins is regulated by estrogen and/or progesterone in a temporal manner [48-50]. Progesterone-dependent growth suppression in the uterus is considered to be mediated by decreased cdks activity via decreased levels

of cyclins and increased association of cdks with CKI [51]. It is reported that progesterone inhibition of uterine epithelial proliferation is mediated by the inhibition of nuclear translocation of cyclin D1 and the association of cdk4 with cyclins A and D-dependent cdk2 activity [52]. In mice, the expression of cyclin D3 is up-regulated at the stromal cell at implantation site and is associated with cell proliferation [53-55]. Furthermore, the expression of cyclin D3 is associated with the polyploidy cells that are defined as terminally differentiated stroma. The significance of polyploid stromal cells is still not clear but it is believed that the life span of decidual cells is limited during pregnancy and that makes room for growing embryo. In addition, one of many uterine functions is to support embryo growth which requires increased protein synthesis from decidual cells. The tight coordination of the cell cycle molecules appears to be critical for uterine cell proliferation and decidualization.

Role of prostaglandins in decidual induction: In rodent, convincing evidence indicated that that the mediators for the decidualization are prostaglandins (PGs) and platelet aggregating factor (PAF). In rats, decidualization can be blocked or its onset can be delayed by inhibiting prostaglandin E2 (PGE2) production [56]. PTGS1 and PTGS2 enzyme mediate PG synthesis and were found in endometrial during embryo implantation in rats [57]. This prostaglandin synthase is present initially in epithelial cells and then in sub epithelial stromal cells [57]. PTGS1 is a constitutive form and PTGS2 is an induced form. Decidual stimulus rapidly induces the expression of PTGS2 but not PTGS1 gene, and null mutation of *Ptgs2* gene prevents decidualization [58]. Further study reported that PGI2, acting through PPARβ is the prostanoid that regulates decidualization in *Ptgs2*-knockout mice [59]. In addition, inhibition of PTGS2 or null mutation of the PTGS2

enzyme impaired implantation [60]. Further studies in which wild-type blastocysts were transferred into *Ptgs2*-deficent uterus indicated delayed decidualization, without substantial effect on implantation , suggesting that PTGS1 may function backup of PTGS2 [61]. Support for this view come from studies in which *Ptgs1* and *Ptgs2* mutant mice were treated with the inhibitors of alternative isoforms, resulting in the complete abrogation of implantation and decidual response [62].

cAMP signaling pathway: Although the postovulatory rise of progesterone control the differentiation of endometrial stromal cell, the first decidual transformation is only apparent at the mid-secretory phase of menstrual cycle indicating that the expression of the decidual specific genes is unlikely under direct control of progesterone receptor (PGR). Overwhelming evidence indicated that the initiation of the decidual process is dependent upon the increased cellular cAMP and sustained activation of the PKA pathway [11]. ESCs cultured with P4 alone or combination of E2 and P4 for 6-8 days will trigger the expression of decidual makers, this response is mediated by a gradual increase in intracellular cAMP and abrogated in the presence of PKA inhibitor [63]. During secretary phase, local factors like PGE2, relaxin and corticotrophin releasing hormone are capable of increasing cAMP level in endometrial stromal cells. Previous studies also reported the expression of these ligands and their respective receptors in the endometrium [64-66]. Furthermore, the ability of these local factors alone or combination with progestin to initiate decidual reaction has been demonstrated [11].

Transcription factors immediately downstream of cAMP signaling: in PKA pathway, binding of cAMP to two regulatory subunits leads to release and activation of the two catalytic subunits of the holoenzyme. The catalytic subunits then phosphorylate

cytoplasmic or nuclear target molecule. Among the major nuclear targets are the cAMP response elements binding protein (CREB) and the related cAMP response element modulator (CREM) [67]. CREB and CREM after PKA phosphorynlation will bind to their cognate DNA sequence (cAMP response element) in cAMP regulated genes and activate transcription [68]. Notably, *Hand2* contains a conserved CREB binding site in its proximal promoter [69], it indicates that *Hand2* may be regulated by cAMP.

Subsidiary transcription factors: Amongst many transcripts that are regulated in decidual process, relatively few encode transcription factors. Most of the transcription factors implicated in the regulation of decidualization have been identified by promoter analyses of decidual marker genes such as PRL and IGFBP1[70]. The most comprehensively studied transcription factor in decidualization is the forkhead transcription factor FOXO1A. FOXO1A is a direct downstream target of PI3K signaling pathway which is stimulated by a number of growth factors and insulin like growth factors [71]. FOXO1A is markedly up-regulated upon decidualization and involved in regulating the decidual markers like PRL an IGFBP1 [72-74]. However, FOXO1A is not expressed in mouse decidual tissue preventing further study in rodents. Previous studies reported a number of transcription factors besides FOXO1A, such as HOXA10, HOXA11, ETS1, in regulating the decidual process [70, 72, 73]. However, the detail mechanism and the interaction of these signaling pathways are not known.

1.3. Basic helix-loop-helix transcription factors

The transcription of gene in eukaryote cells is regulated by enzymes and the interaction of transcription factors on specific regions of target gene. These DNA-binding transcription factors are classified into a number of groups, each defined by shared

sequence, and presumably structure, motif. One of such a motif is the helix-loop-helix (HLH), which was first identified in an immunoglobulin enhancer-binding polypeptide [75]. The basic helix-loop-helix family of proteins is composed of two classes (A and B) of transcription factors important in variety of biological processes, including cell growth and differentiation [76]. Class A bHLH transcription factors are also referred as E-proteins which are ubiquitously expressed in variety of cell types and function by forming homo- and heterodimers with other bHLH transcription factors [77]. Class B bHLH transcription factors are expressed in a tissue-specific manner and mostly contribute to cell differentiation and tissue specific. Class B proteins generally function as heterodimers with E-proteins and bind to the palindromic DNA sequence CANNTG (E-box motif) to regulate expression of their target genes [77, 78].

Expression of HLH proteins are carefully regulated in the development of many tissues. For example, the expression of 2 bHLH transcription factors, HAND1 and HAND2 are critical for vertebrate heart development [79-81]. These two highly conserved transcription factors are the first genes expressed in developing ventricular chambers [82, 83]. Mice lacking *Hand2* resulted in embryo death due to cardiac defect [81]. Although the role of bHLH transcription factors in organogenesis is uncontroversial, how they are regulated in the adult tissue is little known. Previously, Bany and Cross showed that *Hand2* is expressed in the uterine decidual tissue [28]. Recently, there is evidence that *Hand2* is critical for uterine receptivity [84]. However, the detail function of *Hand2* in the uterine receptivity and in regulating decidual reaction is not clear. The further study to investigate the expression of HAND2 in mouse

implantation as well as the control factors of HAND2 gene in human cell culture will shed light on the roles of HAND2 in implantation and decidualization.

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