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Developmental regulation of decidual cell polyploidy at the site of implantation

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1. ABSTRACT

Polyploidy has been reported in several animal cells, as well as within humans; however the mechanism of developmental regulation of this process remains poorly understood. Polyploidy occurs in normal biologic processes as well as in pathologic states. Decidual polyploid cells are terminally differentiated cells with a critical role in continued uterine development during embryo implantation and growth. Here we review the mechanisms involved in polyploidy cell formation in normal developmental processes, with focus on known regulatory aspects in decidual cells.

2. INTRODUCTION

Infertility affects approximately 15% of couples during the reproductive years with implantation failure contributing largely to this statistic (1). In early pregnancy, ovarian estrogen and progesterone drive uterine differentiation, which is critical for embryonic growth and successful implantation (2, 3). Uterine differentiation in the receptive uterus is characterized by transformation of uterine stromal cells into decidual cells (decidualization) Defective decidualization at the site of implantation is one mechanism responsible for implantation failure resulting in infertility (4-6). Development of functional decidua begins with widespread stromal proliferation, followed by localized differentiation into these specialized cells, and subsequent development of polyploidy cells (7, 8). Polyploidization is the hallmark of mature decidual cells characterized by large mono- or binuclear cells with multiple copies of chromosomes and has been well recognized in rodents (9-11) and recently in the humans (Hirota Y and Dey SK, unpublished observation). The physiologic significance of this process in decidual cells appears to be crucial for successful embryo implantation and the support of embryo growth during the early pregnancy (1).

The development of polyploid cells is widely reported in nature occurring in a variety of plant and animal models. In mammals, several cells including hepatocytes, cardiac myocytes, arterial smooth muscle cells, megakaryocytes, trophoblasts, and decidual cells can all acquire varying degrees of polyploidy during their cell

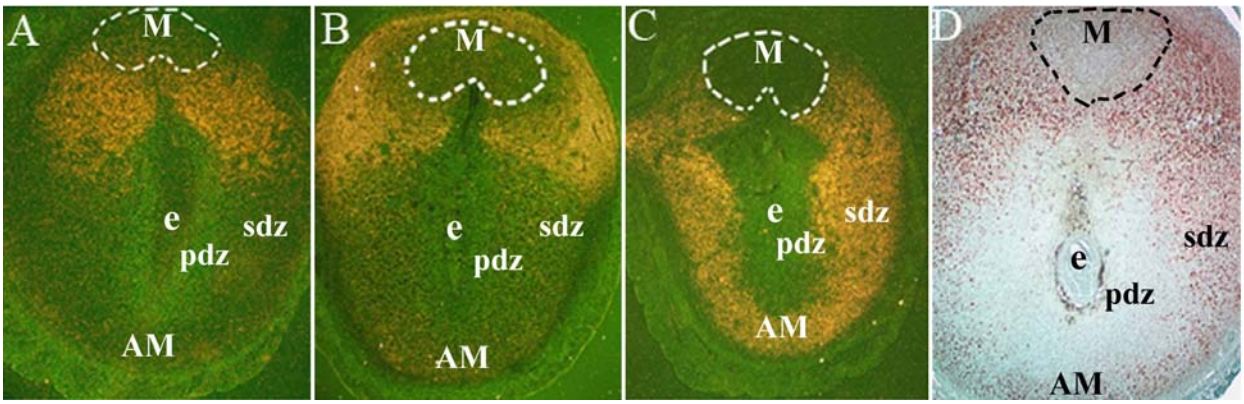

Figure 1. Regional distribution of expression for up-regulated polyploidy genes during decidualization at the site of implantation. *In situ* hybridization: Expression of *Nox4* (A), *Nsbp1* (B), and *Tdo2* (C) genes at the embryo implantation sites on day 7 of pregnancy. Dark-field photomicrographs of representative cross-sections hybridized with antisense probes are shown at 40X. Immunohistochemical analysis: Localization of CyclinD3 (D) at the embryo implantation sites on day 8. M, mesometrial pole; AM, anti-mesometrial pole; e, embryo; pdz, primary decidual zone; sdz, secondary decidual zone. (Reproduced with permission from, ref# 49).

lifespan (6, 12-14). In addition, various biologic mechanisms occur in conjunction with polyploidy development, including cellular differentiation (8, 15), tissue regeneration (16), nutritional/metabolic activity (16, 17), and embryo implantation (1). The developmental mechanisms and function of polyploidy still remain largely unknown. Loss of regulation during the cell cycle is one possible mechanism for polyploidy development, by which the cell undergoes continuous DNA synthesis in the absence of cytokinesis resulting in formation of mono- or binucleated cells, containing DNA with multiple copies of the haploid complement (6, 12-14, 17). Here we focus on the mechanisms involved in polyploid cell generation as well as the function of polyploidy in decidualization.

2.1. Characteristics of polyploid cells

Several characteristics are endemic to polyploidy cells including cell size, DNA content, and metabolic activity. Here we briefly review these characteristics. Polyploidy commonly is associated with the termination of a highly proliferative phase in many tissues both during pathologic and normal physiologic processes including the uterine decidual bed (7, 8, 12). Polyploidization may be developmentally advantageous during rapid growth, modifying the cell cycle to utilize less energy expenditure by increasing cell volume and to bypass large cell surface area needed for several generations of cell division (16). Polyploid cells have increased cell size proportional to increase in DNA content (18). The cell size appears to be a result of the rate of both cell growth and cell division. Furthermore, the cell volume is similar between binucleated 2 x 2n and mononucleated 4n or binucleated 2 x 4n or mononucleated 8n (19), consistent with evolutionary conservation in eukaryotes between DNA content and cell volume (20). Polyploid cell formation from controlled mechanisms in normal physiology has specific characteristics that differ from those processes causing polyploidization in pathologic processes. Polyploid cells resulting from endoreplication produces cells where the DNA content is in integral multiples of the normal diploid, where abortive cell cycle processes associated with tumor formation produce cells with DNA content that varies continuously from 2N to significantly greater values (21). Moreover, terminally differentiated polyploid cells from endoreplication maintain viability for long periods of time whereas polyploidy from DNA damage activates apoptotic pathways (22). These processes characteristic to polyploid cells are likely implemented in time of rapid cell growth (i.e. decidualization) or when energy availability is limited; nevertheless adaption of cell growth and cell cycle regulation appear to be well controlled for directed purpose specifically to support embryo implantation within the endometrium.

2.2. Characteristics of decidual polyploidy

At the site of the implanting blastocyst, decidualizing stromal cells undergo extensive proliferation and differentiation. In the mouse model, localized stromal cell differentiation into specialized cells (decidual cells) occurs on day 5 of pregnancy (day 1 = vaginal plug) with polyploidization occurring days 6-8 of pregnancy (7, 8). Differentiated stromal cells adjacent to the implanting blastocyst form the primary decidual zone (PDZ) at the antimesometrial pole by the afternoon on day 5. The PDZ is composed primarily of epithelioid cells and is avascular in nature (23). By day 6, the secondary decidual zone (SDZ), comprised of proliferating and differentiated polyploidy stromal cells, forms adjacent to the PDZ. By day 7 afternoon, the SDZ is fully developed and polyploidy development now gradually spreading, occurring at the antimesometrial pole and at the lateral junctional region between the mesometrial and antimesometrial poles (Figure 1) (8). By day 8, the cells comprising the PDZ have progressively degenerated by apoptosis and mostly disappeared. The mesometrial pole, between day 7 and 8, is characterized by stromal cell proliferation and differentiation, but forms a non-polyploid decidual zone prior to placentation. Cell-cycle molecules, growth-factors, signaling mediators, and homeobox transcription factors have been shown to regulate decidualization as well as

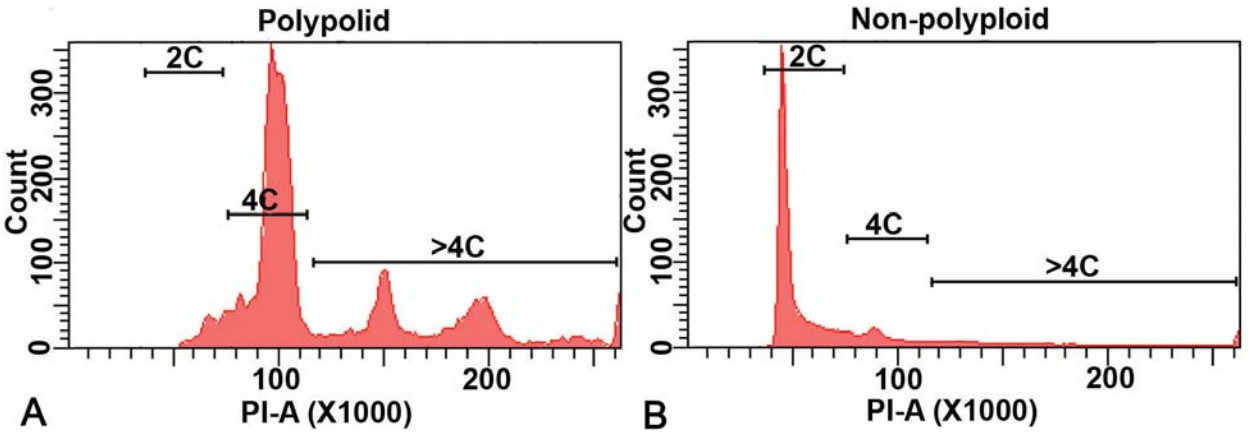

Figure 2. Analysis of polyploid and non-polyploid cells. Deciduomal cells collected on day 7 of pseudopregnancy. Flow cytometric analyses of the DNA content for a representative polyploid (A) and non-polyploid (B) population are shown. Note: The polyploid fractions are enriched with DNA content > 4N, while the non-polyploid fractions are primarily devoid of cells with DNA content > 4N. (Reproduced with permission from, ref# 49).

decidual cell polyploid formation (4-6, 24-26). In decidual cells, polyploidy cells have DNA content >4N, while non-polyploidy cells are composed of DNA content of 2N (Figure 2). Recently, differential regulation of genes were investigated to characterize gene expression patterns in decidual polyploidy cells as compared to nonpolyploidy decidual cells by microarray studies (Figure 3) (27). The physiologic importance of decidual polyploidy remains unclear however it is speculated that polyploidy formation limits the lifespan of the decidual cells to allow for growth of the implanting embryo as well as increased quantity of protein synthesis from endoreplication that is needed for embryo growth (6).

3. MECHANISMS OF POLYPLOIDIZATION

Within the normal cell cycle, the cell must obtain a complete copy of chromosomes with each cell division; otherwise the genomic stability within the cell can be compromised. Polyploidy occurs from deviations from the normal G1-S-G2-M cell cycle that allows genome replication without cell division (16). Several general mechanisms are considered for polyploidy cell formation occurring in diploid species, including: cell fusion, endoreplication (endomitosis), and an abortive cell cycle (20). In normal mammalian developmental processes, endoreplication appears to be the main mechanism leading to polyploidy generation (17). Abortive cell cycle involving defects in DNA replication, separation of sister chromatids, mitotic spindle checkpoints, and cytokinesis which leads to genomic instability (both polyploidy and aneuploidy) typically involved in pathologic states (20). However, polyploidy occurring in normal hepatocytes development may result from both incomplete cell division as well as endoreplication (12, 28). Here we review the proposed mechanisms involved in polyploidy cell generation most likely involved in decidual polyploidy formation by focusing on those processes involved in normal biologic processes.

3.1. Regulatory mechanisms of the endocycle

Endoreplication generates polyploid cells by a regulated cycle uncoupling DNA replication from cell division (17). In proliferating cells, the cell cycle is regulated by Cyclin-dependent kinases (Cdks), determining entry into both mitosis and S phase. Cdk1 is an important mediator of mitotic control. A and B type Cyclins and Cdc25 type phosphatases are activators of Cdk1 for mitotic phase regulation (29). Some endoreplicating cells may bypass mitosis, in these cells expression of Cdk1 and/or activators of Cdk1 may be diminished or aberrant indicating that Cdk activity may determine the degree of mitotic function within the endoreplicating cell (17). Initiation and progression of the S phase is regulated by Cdk2 by forming complexes with Cyclins A and E. The D type Cyclins with Cdk4 and Cdk6 mediate G0 to G1 transition. Fluctuations in Cyclin/Cdk levels through the normal cell cycle have been observed to maintain normal DNA replication and equal cell division (30). Low Cdk activity during G1 allows for formation of pre-replicating complexes (preRCs) necessary to signal that another round of DNA replication can be initiated. Cdks activity then increases in S and G2 phases inhibiting preRCs binding therefore blocking DNA replication during these cycles. During endoreplication, it is thought that Cdk activity oscillates between low and high levels to allow for multiple rounds of DNA replication and generation of polyploidy.

Cdk inhibitors (Ckis), which bind to and inhibit Cdk activity, are likely candidates for other regulators of endoreplication cycles by contributing to oscillations of Cdk/Cyclin levels. In addition, Ckis likely protect against inappropriate re-replication (29). Several Ckis, p57, p27, and p21, in vertebrates have been found to control cell cycles via inhibition of Cdk phosphorylation. Levels of Ckis oscillate during the cell cycle as well in order to allow for endoreplication (31). Overall, the specific interactions and expression levels of Cdks, Cyclins, and Ckis are important to the development of polyploidy in decidual cells as well as other cell types (Table 1).

Table 1. Cell cycle regulatory proteins involved in polyploidy in normal biologic processes

Category	Regulatory protein	Expression profile during endocycle	Stage of cell cycle	Cell types
Cdks	Cdk 1	Down-regulated	M	Decidual cell, trophoblasts, megakaryocytes
	Cdk2	Up-regulated	S,G2	Decidual cell, trophoblasts, megakaryocytes
	Cdk4	Up-regulated	G1	Megakaryocytes
	Cdk6	Up-regulated	G1, S, G2	Decidual cell, megakaryocytes
Ckis	p21	Up-regulated	G1, S, G2	Decidual cell
	p57	Up-regulated	G1	Trophoblast
Cyclins	Cyclin A	Up-regulated	S, G2	Decidual cell
	Cyclin B	Down-regulated	M	Decidual cell
	Cyclin D3	Up-regulated	G1, S, G2	Decidual cell, trophoblasts, megakaryocytes
	Cyclin E	Up-regulated	G1/S transition	Decidual cell, trophoblasts, megakaryocytes

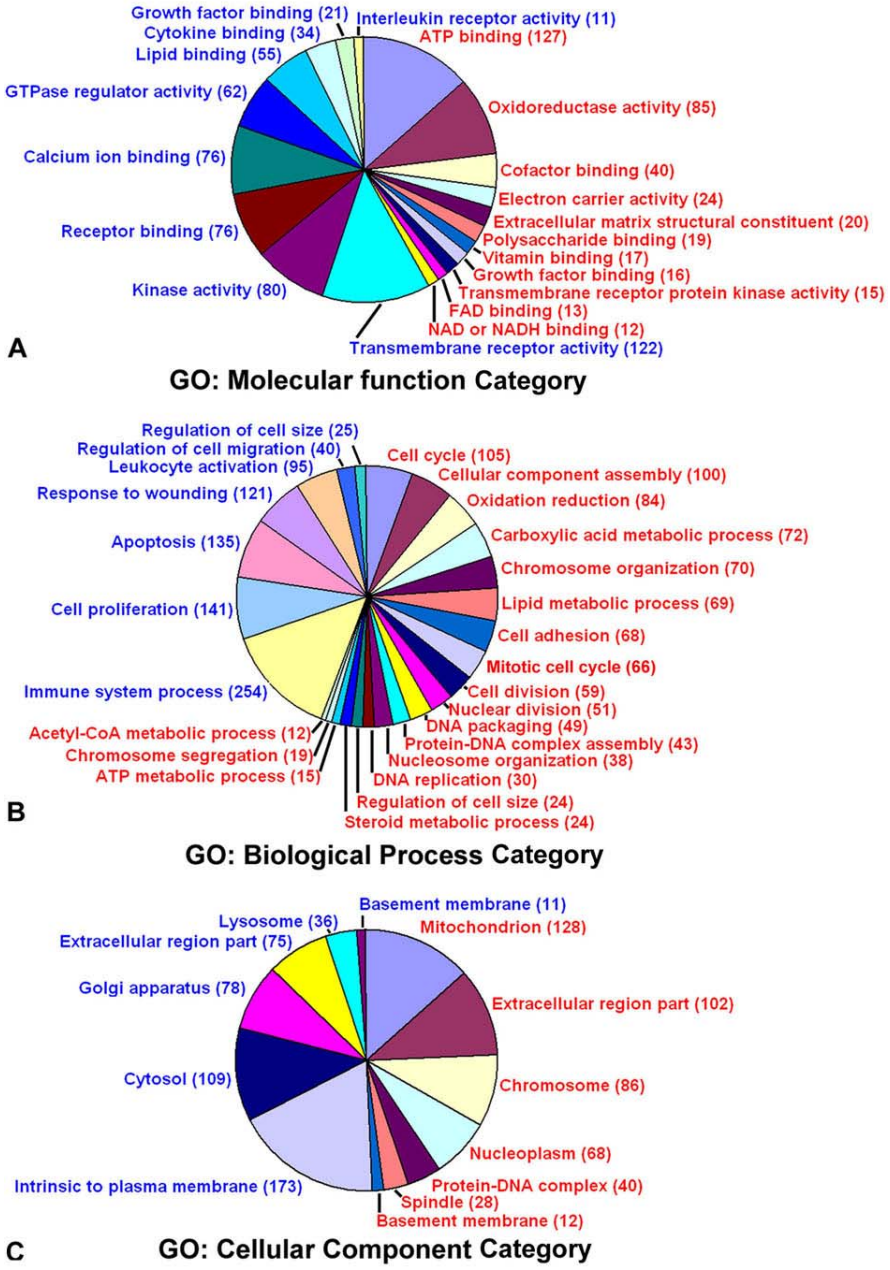

Figure 3. Functional categorization of differentially expressed genes in the polyploid decidual cells. The up-regulated (red) and down-regulated (blue) genes are defined by the gene ontology (GO) terms: molecular function, biological processes, and cellular component categories. The number within the parenthesis represents the total genes modulated under a category. (Reproduced with permission from, ref# 49).

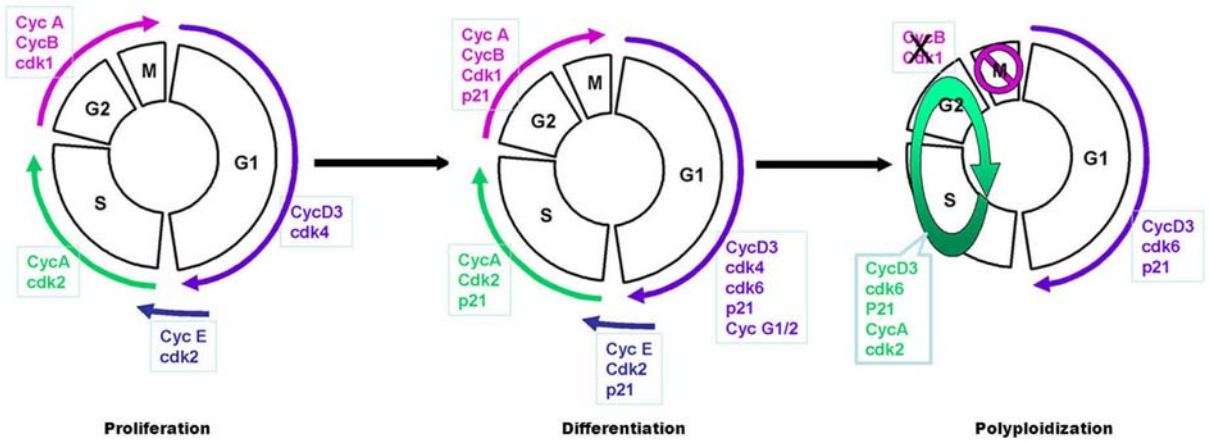

Figure 4. A proposed model for stromal cell proliferation, differentiation, and polyploidization. (Reproduced with permission from, ref# 6).

3.1.1. The endocycle of decidual cells

The polyploid decidual cells are thought to be terminally differentiated cells that develop via endoreplication cycle with repeated rounds of DNA replication without cytokinesis (6). The cell cycle is tightly regulated during decidualization with interplay between Cyclins, Cdks, and Ckis controlling proliferation and differentiation (Figure 4). More specifically, CyclinD3, Cdk4, Cdk6, and the Ckis p21 and p57 expression appear to play a role in directing decidualization (8). CyclinD3 has low expression within murine stromal cells on the morning of day 4, however its expression is dramatically up-regulated in decidualizing stromal cells following the initiation of implantation (32) whereas CyclinD1 and D2 have low expression prior to and during decidualization (8). CyclinD3 plays an important role in stromal proliferation, differentiation, and polyploidy formation in a stage-specific progression with temporal relationship with blastocyst implantation. CyclinD3 co-expression with Cdk4 is up-regulated in proliferating decidual cells in both the PDZ and the SDZ; however after proliferation is complete, Cdk4 down-regulation occurs in these zones (8) suggesting proliferation is controlled by CyclinD3 and Cdk4 co-activity. Furthermore, in polyploidy decidual cells we observed the overexpression of CyclinD3 with p21 and Cdk6, but not Cdk4, indicating that a complex of CyclinD3/Cdk6/p21 is implicated in polyploidy cell formation in decidual cells. Investigation of other Cyclins and Cdks during decidual polyploidy found that a large number of polyploidy cells demonstrate a heightened expression of CyclinA but not of CyclinB. Furthermore, Cdk1 and Cdk2 have similar expression patterns as CyclinB and A, respectively. Elevated CyclinE expression was also noted in polyploidy cells in the SDZ on days 6 and 7 of pregnancy. Together suggesting that CyclinB and Cdk1 down-regulation as well as up-regulation of CyclinA, CyclinE, and Cdk2 are also important in the regulation of signaling in the decidual cell endoreplication cycle (8). Although several cell cycle regulators are involved in uterine decidualization, CyclinD3 is the most investigated with studies demonstrating that other regulators of decidualization i.e. HB-EGF (heparin binding EGF-like growth factor) (33), DEDD (death-effector domain-containing protein) (1), Hoxa-10 (homeobox A10) (7, 32), Stathmin (34), IL11/IL11ra/BIRC5 (35), BTEB1 (basic transcription element-binding protein-1) (36), utilize CyclinD3 as a downstream controller of uterine decidualization and polyploidy formation.

HB-EGF, an early marker of implantation (37), controls stromal cell polyploidy and binucleation by interacting with cell cycle regulatory molecules, specifically HB-EGF causes a marked overexpression of CyclinD3 in decidual cells (33). Polyploid decidual cells in *in vitro* culture demonstrated co-localization of CyclinD3/Cdk6/p21 in HB-EGF cultured cells but not in cells cultured in the absence of HB-EGF. In addition, *in vivo* experiments using HB-EGF-soaked beads transferred into pseudo-pregnant mouse uteri demonstrated presence of large mono- or binucleated cells in SDZ with expression of CyclinD3/Cdk6/p21 co-localized activity but this was not seen with BSA-soaked control beads. In addition, treatment of uterine stromal culture cells with antisense CyclinD3 and HB-EGF was completely inhibitory to polyploidy formation, and *in vivo* administration of CyclinD3 antisense adenovirus demonstrated reduced uterine weights on both days 7 and 8 of pregnancy (33). The CyclinD3 deficient mice also result in decidualization defect at the site of implantation with sub-fertility phenotype (6). Similarly, the loss of HB-EGF in mice also exhibits defects in the aspects of decidualization phenotype in early pregnancy (38). Together, these results suggest that CyclinD3 is crucial to stromal cell polyploidy formation regulated by HB-EGF both *in vitro* and *in vivo* (33).

DEDD, a member of DED-containing protein family, is found to regulate a variety of cellular signaling pathways, and has recently been implicated in uterine decidualization in the murine model (1). DEDD is associated with CyclinB1/Cdk1 activity; specially decreasing the activity of Cdk1 allowing for sufficient ribosomal RNA/protein synthesis and cell growth prior to cell division by inhibiting Cdk1 regulated mitotic progression (39, 40). DEDD also interacts with Akt and S6K1 within the PI3K signaling cascade (41, 42). *Dedd*

null mice are completely sterile, and the infertility is likely secondary to decidualization defects. *Dedd* null mice exhibit normal implantation as compared with wild-type animals with similar weights on day 4.5 of pregnancy. However, the number of viable embryos rapidly declined starting on day 5.5, and by day 9.5 no living embryos were detected in *Dedd*$^{-/-}$ uteri (1). In addition, in *Dedd* null mice both immunostaining and mRNA expression levels for common decidualization markers were significantly reduced starting on day 5.5. Attenuation of decidual polyploidy was also noted in *Dedd* null mice. Expression of both Akt and CyclinD3 levels were reduced in these mice resulting in reduced polyploidy formation. The overexpression of both Akt and CyclinD3 in *in vitro Dedd* null stromal culture resulted in an increased proportion of polyploid cell formation. Additionally, DEDD/CyclinD3/Akt were co-localized in the decidual zones *in vivo*, with DEDD co-precipitated with CyclinD3 as well as CyclinD3/Cdk4 and CyclinD3/Cdk6. These finding suggest that DEDD plays an important role in uterine decidualization and polyploidization possible via Akt regulation as well as direct interaction with key cell cycle regulators (1). Mediators of cell cycle regulation play a significant role in stromal cell decidualization and polyploidy formation; however a complete understanding of all the mechanisms involved in decidual polyploidy must still be determined.

Hoxa-10, a member of the homeobox family of transcription factors (43), exhibits abundant expression in the uterine stroma during the receptive phase. The expression pattern is consistent with the status of proliferation and differentiation with the onset of decidualization (44). The loss of *Hoxa-10* in mice have resulted in a complete failure of pregnancy, with a defective decidualization phenotype (45), and studies have shown that CyclinD3 expression remains downregulated in Hoxa-10$^{-/-}$ uteri during the progression of decidualization (32, 33). Overall, these results suggest that the loss of Hoxa-10 impairs decidualization primarily due to the deficiency of CyclinD3 at the site of implantation. Stathmin, a cytosolic phosphoprotein, has been shown to express in the antimesometrial decidual bed of the implantation site in mice and its deficiency in mice showed downregulation of CyclinD3 in the decidual bed with reduced fertility (34). BTEB1, a member of the Sp/Krüppel-like family of transcription factors, has been shown to interact with PR isoforms and express during decidualization in a PR-A-dependent fashion (36). Interestingly, *Bteb*$^{-/-}$ mice also show sub-fertility with predominant downregulation of CyclinD3 and Hoxa10 during decidualization (36). Moreover, recent studies also provided evidence that the critical decidualization signal via IL11/IL11ra (46, 47) also utilizes cyclin D3/p21 in conjunction with BIRC5 as downstream targets at the site of implantation (35). Despite the existing link of the above regulators with CyclinD3/p21, their roles in polyploidy development remain unknown and should be the subject for future study.

Additionally, uterine-specific *p53* (a tumor suppressor gene) deficiency demonstrated impaired decidualization. In these mice, an elevated expression of p21 was noted with an increase in the number of decidual polyploidy cells. Typically p21 is up-regulated by p53 after DNA damage, but p21 can also be induced independent of p53. These results suggest that the loss of p53 enhances decidual cell polyploidy formation via stimulation of endocycle pushing these cells to terminal differentiation (48).

Recently, gene expression profiling comparing decidual polyploidy and non-polyploidy cells revealed a large group of genes (2222 genes total) that are differentially expressed, which suggests that a dramatic alteration of gene expression is necessary to potentiate the transition from a non-polyploidy state to a polyploidy state (49). Genetic expression analysis revealed several altered cell cycle genes, specifically 105 up-regulated and 11 down-regulated genes were found, confirming the importance cell cycle regulatory molecules in decidual cell polyploidy. A significant number of genes involved in the mitotic phase (72 total) were altered as well as p57, an inhibitor of Cdk1, which is associated with polyploidy in trophoblast giants cells (21). Several genes involved in nuclear division were also up-regulated and several genes related to bi-nucleated polyploidy were noted. Specifically, Td02 and Nsbp1 were detected in the bi-nucleated polyploidy cells within the decidual bed (49). Several minichromosome maintenance protein complexes (Mcm) that serve as eukaryotic helicase in DNA replication were up-regulated in polyploid decidual cells including Mcm2-5, and Mcm7.

Overall, endoreplication thus occurs with modification of the cell cycle to promote controlled repeat DNA replication producing polyploid cells. Multiple mechanisms in various species exist to allow for endoreplication to occur; and different cell types utilize different mechanisms. Although the specific mechanisms utilized by decidual cells remains unclear, understanding mechanisms in endocycles of other cells may help to uncover the gaps in knowledge of decidual cell polyploidy.

3.1.2. The endocycle of other cells

In Drosophila, continuous expression of CycleE/Cdk 2 or CyclinE genetic mutation results in cessations of endocycles (50-52). While triggering S phase, CyclinE/Cdk2 activity is also responsible for ensuring that only one DNA replication occurs during the S phase by directing separation of pre-RCs from origins of replication during oscillations of activity (53). Additionally, Cdk4 is the target of CyclinD3 and directs cell growth in Drosophila (54).

Several mammalian cells utilize the above mechanisms to undergo endoreplication during normal physiologic processes. CyclinE activity is required for endoreplication in rodent trophoblasts and megakaryocytes (55) with the Cyclin E/Cdk2 complex playing a major role in both mammal and insect models (16). Megakaryocytes require CyclinD3 for polyploidy formation, with CyclinD3 overexpression causing increased polyploidy in these cells (56). Reduced CyclinD3 levels show inhibition of polyploidy with thrombopoietin controlling expression of CyclinD3 in these cells (56). Cdk4 and Cdk6 are possibly

the downstream target of CyclinD3 in megakaryocytes, however, this has not been extensively investigated. In megakaryocytes, cells complete anaphase A with separation of sister chromatids but will not complete anaphase B. This process therefore results in replicated chromosomal copies within the same nucleus, skipping cytokinesis completely (17). Decreased levels of CyclinB and CyclinB/Cdk1 have been found in endocycles of these cells, and excess degradation of CyclinB may drive deviation from anaphase B and cytokinesis (57).

Mammalian trophoblastic cells undergo cyclic DNA replication with certain mitotic features. In these cells, during endocycles, CyclinD1 is induced, high levels of CyclinE and A are noted, but CyclinB levels are diminished consistent with other findings in mammalian endoreplication cycles (58). Endoreplication is severely impaired in CyclinE deficient trophoblastic mouse cells by failing to incorporate MCM proteins into DNA replication origins (55). In addition, P57 levels in rodent trophoblasts are up-regulated in the G-phase and it acts via Cdk1 suppression to stimulate endoreplication (21).

3.2. Other mechanisms in polyploidization

3.2.1. Spindle checkpoints

Mitosis is the final stage of the cell cycle. During this stage, duplicated chromosomes from the S phase are separated into two equal daughter cells. For equal segregation of chromosomes, which have random distribution within the nucleus during interphase, alignment at the spindle equator in mitosis must occur prior to the initiation of sister chromatid separation. The spindle checkpoint is a regulatory mechanism that delays entry into anaphase until each chromosome is on the way to the spindle equator and all chromosomal kinetochores are attached to the spindle (59). Failure of checkpoint mechanisms results in polyploidy and aneuploidy that have been well-studied in tumorigenesis, but also occurs in some normal cell processes for endoduplication (59).

The spindle checkpoint is activated in the presence of kinetochores unattached to the spindle microtubules to prevent entry into anaphase. Mad (mitotic arrest deficient) 1, 2, 3, Bub (budding uninhibited by benzimidazole) 1, and 3 are identified genetic factors involved in spindle checkpoint. Mad2 is one of the most important components of checkpoint activation (60, 61) by forming a complex with Cdc20 (cell-cell division protein 20) preventing activation of Cdc20-APC/C (Anaphase Promoting Complex or Cyclosome) which activates Separase to cleave the two sister chromatids (62). Overexpression of *Mad2* can delay anaphase, but in eukaryotes this overexpression induces tetraploidy likely because cells eventually transition from mitosis without sister chromatid separation (59). In addition, transgenic mice with overexpression *Mad2* demonstrated chromosome instability with resulting tetraploidy (63). In this regard, a recent study by our group demonstrated in decidual polyploidy cells up-regulation of several genes involved in nuclear division (49). Among these were genes encoding proteins such as Mad2L1, Mad2L2, Bub1, and Bub1B which are involved in spindle assembly checkpoint and maintenance of chromosomal stability (64).

3.2.2. Cytokinesis failure

Cytokinesis occurs by sequential progression through four stages: determining the cleavage plane, ingression of the cleave furrow, formation of the midbody, and abscission (65). Improper completion of any stage can lead to failure of cytokinesis resulting in polyploidy formation, and some proteins regulate multiple steps in cytokinesis thus alterations in these proteins may make cells prone to incomplete cytokinesis. Failure of cytokinesis has historically been associated with production of tetraploid cells that give rise to tumors; however recently cytokinesis failure has been associated with normal physiologic processes (28, 66). The RhoA pathway is essential for efficient and correct furrow formation to occur in animal models (65, 67). Localized activation of RhoA is vital to microtubules in delivering signals in the furrow; aberrations of these signals by spindle disruption can lead to cytokinesis failure. Failure during the initial stage of cytokinesis by disrupted spindle elongation or positioning has recently been reported in normal physiology of hepatocytes (28, 66). In rat hepatocytes, failure of cytokinesis in diploid, mononucleated cells results in polyploidy cells secondary to failure of contractile ring formation (28) during mitosis and thus cells fail to undergo anaphase (66). Processes in cytokinesis fall under control of Cdk regulators (68). Cdk1 inactivation must occur for progression of cytokinesis, whereas Polo kinase and Aurora B kinase must be activated following Cdk1 inactivation to stimulate cytokinesis (65). Cdk1 inactivation may be the trigger for cytokinesis initiation via regulation of the RhoA pathway; Polo kinase also functions by regulating the Rho A pathway. Interestingly, some of the regulators have been found to upregulated in polyploid decidual cells (49), however, the role of these specific regulators in decidual cells is unclear and require further research to better understand the mechanisms in decidual polyploidy.

4. FURTHER CHARACTERIZATION OF DECIDUAL POLYPLOIDY

Uterine stromal cell polyploidization is crucial in directing decidualization and early implantation within the mouse model (1, 48). In our recent study, we utilize pure polyploid decidual cells as a model for further characterization of mouse decidual cell polyploidy formation by characterizing gene expression patterns within this model (49).

4.1. The mitochondrial role in decidual polyploidization

The development of polyploidy via endoreplication occurs in various cells during times of tissue regeneration and stress to promote growth and proliferation. During time of oxidative stress there is an increase in ROS production via Nox4 (NADPH oxidase 4) enzymatic activity (69-72). Hyperactivation of mitochondrial activity stimulated increased ROS production (73); and Nox4 has been shown to be associated with polyploidy development (69). High energy driven mitochondrial activity has been shown to be important in

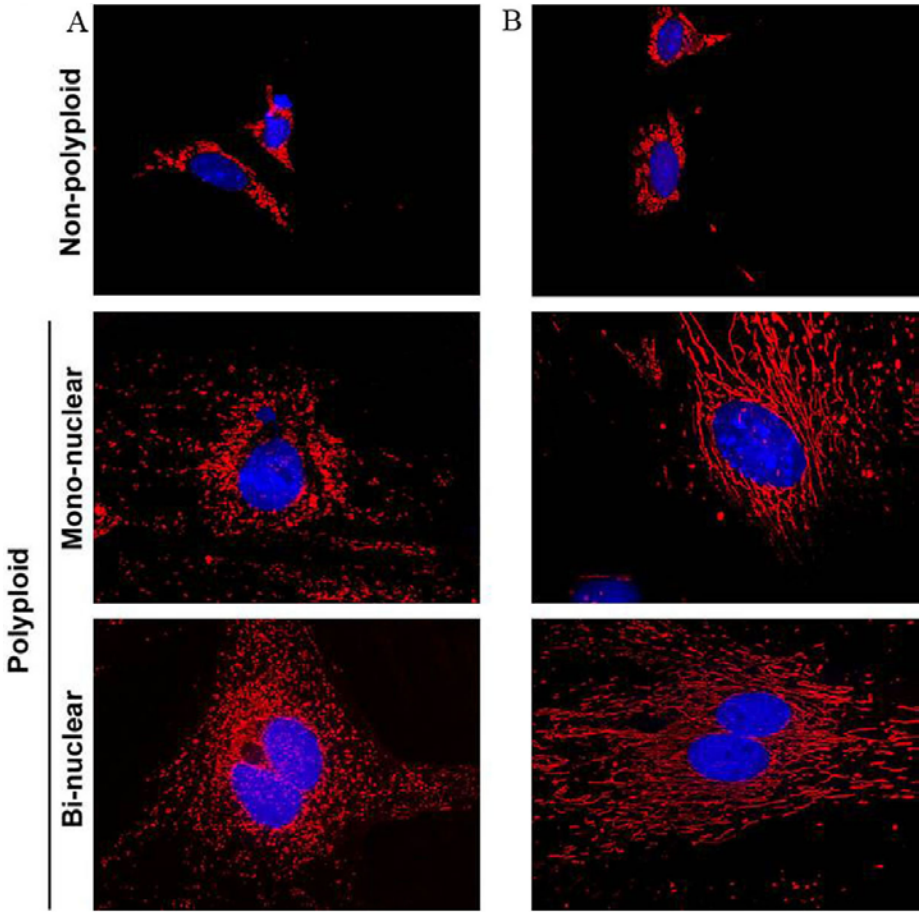

Figure 5. Analysis of mitochondria in relation to decidual cell polyploidy. Mitochondrial mass analysis by confocal microscopy. (A) Pure polyploid and non-polyploid decidual cells isolated from day 7 deciduomal tissues. (B) Day 4 uterine stromal cells in culture were used to induce decidualization *in vitro*. Polyploid and non-polyploid cells were collected on day-5 following the induction of decidualization. Both cell populations derived *in vivo* and *in vitro* were cyto-spun onto slides and analyzed by staining with Mitotracker *Red*. (Reproduced with permission from, ref# 49).

polyploid decidual cell development (49), in order to support both uterine differentiation and embryo implantation. Global gene expression profiling of polyploid decidual cells, as compared to non-polyploid decidual cells via microarray analysis revealed 7 mitochondrial gene networks including lipid metabolism, carbohydrate metabolism, cellular assembly and organization, genetic disorder, metabolic disease, small molecule biochemistry, and drug metabolism involved in polyploidy development. More specifically, Nox4 was found to be up-regulated in polyploid cells (Figure 1) and is expressed in the decidual bed consistent with polyploidy association (49). Several other identified genes with mitochondria and binuclear characteristic were up-regulated in the decidual bed in a regional pattern, consistent polyploidy expression (49). Marked increase in mitochondrial mass in both binuclear and mononuclear polyploidy cells was noted using confocal microscopy in both *in vivo* and *in vitro* models, as compared to non-polyploid cells (Figure 5). To confirm the role of mitochondria in polyploidization, we uncoupled the respiratory chain and oxidative phosphorylation in decidualizing stromal cell *in vitro*; and found a dramatic inhibition of binucleation and polyploidy (49). In conclusion, mitochondrial activity appears to be crucial to the development of decidual cell polyploidy, an essential process for successful pregnancy (1). Furthermore, abnormal mitochondrial function may impair decidual cell polyploidization causing infertility.

4.2. The role of immune cells and apoptosis in decidual polyploidy

Previously, it has been reported that the implantation site demonstrates a reduction in the immune response (74, 75) suggesting that implantation is protected against maternal immunological response during pregnancy. Although maternal natural killer cells are recruited to the attachment site after embryo attachment (76), other immunological cells including leukocytes and other bone marrow-derived cells migrate from the blastocyst implantation site (75). Consistent with these finding, a large number of immune-related genes are down-regulated within decidual polyploidy cells when compared to non-polyploidy cells (49). Furthermore, it has previously been shown that apoptosis is minimally detected in uterine

polyploid decidual cells (77), and gene expression analysis demonstrated that genes associated with apoptosis are consistently suppressed in decidual polyploidy cells when compared to non-polyploidy cells (49). Overall, it can be suggested that decidual polyploid cells primarily lack apoptosis and immune suppression properties.

5. PERSPECTIVES

Implantation and pregnancy rates even with assisted reproductive technologies still remain disappointingly low likely secondary to poorly understood implantation and decidualization defects. Several restraints exists limiting human uterine-embryo interactions; however animal models with similar processes (i.e. decidualization) to humans provide understanding of mechanisms involved in early embryo-uterine interactions. Continued research of these processes is vital to alleviating problems associated with infertility. Stromal polyploidy formation is a critical step in normal decidualization in both human and rodents. Understanding the physiologic functions of specific factors involved in polyploidy mechanistically via overexpression, silencing, or target gene deletions studies will provide valuable information to improved understanding human decidualization and early pregnancy.

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Abbreviations: PDZ: primary decidual zone; SDZ: secondary decidual zone; cdks: cyclin-dependent kinases; preRCs: pre-replicating complexes; Ckis: cdk inhibitors; DEDD: death-effector domain-containing protein; Hoxa-10: homeobox A10; BTEB1: basic transcription element-binding protein-1; MCM: minichromosome maintenance protein complexes; Mad: mitotic arrest deficient; Bub: budding uninhibited by benzimidazole; APC/C: Anaphase Promoting Complex or Cyclosome; Nox4: NADPH oxidase 4.

Key Words: Embryo Implantation, Decidualization, Polyploidy, Bi-Nucleation, Mitochondria, Cell Cycle, Review

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