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From Cell Biology to Tissue Engineering

Review

The proliferation mechanism of normal and pathological human placentas

Gozde Unek[1], Asli Ozmen[1], Bekir Sitki Isenlik[2] and Emin Turkay Korgun[1]

[1] Department of Histology and Embryology, Medical Faculty, Akdeniz University and

[2]Department of Obstetrics and Gynecology, Antalya Training and Research Hospital, Antalya, Turkey

Summary. The placenta, which is a regulator organ for many metabolic activities between mother and fetus, is critical in influencing the outcome of pregnancy. Therefore, fetal growth is directly related to the placental development. Placental development depends on the coordinated action of trophoblast proliferation, differentiation and invasion. Studies on cell cycle related proteins that control these events are limited. Abnormal placental development is linked to various pregnancy pathologies such as preeclampsia, intrauterine growth restriction, diabetes mellitus and gestational trophoblastic diseases. The cell cycle mechanism of human placenta should be well understood for a healthy pregnancy outcome. Moreover, how cell cycle related proteins that control placental development are affected in pregnancy pathologies is not fully understood yet. Therefore, the aim of this review is to address the currently available knowledge on cell cycle regulatory proteins involved in human placental development and on the expression differences of these proteins in pathological placentas.

Key words: Human placenta, Cell cycle, Cyclins, CKIs, Pregnancy pathologies

Offprint requests to: Emin T. Korgun, Department of Histology and Embryology, Medical Faculty, Akdeniz University, 070079 Antalya, Turkey. e-mail: korgun@akdeniz.edu.tr

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Introduction

The placenta, which is a highly specialized temporary organ regulating many metabolic activities between mother and fetus, critically affects the outcome of pregnancy. Therefore, fetal growth is directly related to placental development. The human placenta is unique in many aspects of its growth and differentiation, including the requirement to invade the uterus for survival. Normal placental development depends on cell proliferation, differentiation and invasion in a proper and simultaneous manner (Genbacev et al., 2000).

In early pregnancy, normal trophoblast development is the key for successful implantation and formation of maternal-fetal interface that facilitates the dialogue between the two organisms. Abnormal placental development has a role in early embryonic death and has been associated with pathologies of pregnancy such as preeclampsia, intrauterine growth restriction, diabetes mellitus and gestational trophoblastic diseases (Maccani and Marsit, 2009).

Placental development involves proliferation and differentiation of the trophoblasts in a manner that is tightly regulated in time and space. An important question in placental biology is what influences the decision of trophoblasts to divide or to differentiate. Several factors are involved to establish a delicate balance between trophoblast proliferation and differentiation, and among them cell cycle proteins play dominant roles during these key transitions (Genbacev et al., 2000). In the human placenta, cytotrophoblasts exit the cell cycle, differentiate and fuse to form the non-mitotic, multinucleate syncytiotrophoblast.

Despite their importance, there is little information about the cell cycle related regulators that provide the synchronization of trophoblast proliferation and differentiation. Moreover, placental abnormalities observed in pathological placentas could be associated with cell cycle and cell cycle arrest mechanisms' alterations which occurred in the pregnancy pathologies. How cell cycle related proteins that control placental development are affected in pregnancy pathologies is not fully understood yet. The present study is designed to review the currently available knowledge on the cell cycle regulatory proteins implicated in the control of proliferation of the human placenta and on the expression differences of these proteins in pathological placentas.

The localization and function of cell cycle related proteins in the human placenta

Cyclins

The eukaryotic cell cycle is regulated by cyclins and cyclin-dependent kinases in a coordinated manner, and controlled by cyclin dependent kinase inhibitors (CKIs) (De Falco et al., 2004) (Table 1). Cyclins are the regulatory subunits of CDKs. In mammals, cyclins can be divided according to the cell cycle phases they are in charge of as cyclin D, -E, -A and -B.

There are three types of cyclin D in mammalian cells: cyclins D1, D2 and D3 (Kumar et al., 2005). They are expressed in different compartments of the human placenta, indicating that cell-type specificity is probably involved in the control of the cell cycle in this tissue. While cyclin D2 was found to be present only in the villous core and cyclin D1 was expressed mostly in cells lining the intravillous vessels, cyclin D3 was found to predominate in the villous trophoblasts but was not expressed in syncytiotrophoblast and invasive cytotrophoblasts of human placenta (DeLoia et al., 1997; Genbacev et al., 2000). In normal placental development, cyclin D3 expression in villous cytotrophoblasts was known to decrease gradually till term (Genbacev et al., 2000). In cultured villous cytotrophoblasts, cyclin D1 was not expressed and cyclin D2 and D3 were expressed but reduced with differentiation (McKenzie et al., 1998).

Two types of cyclin E (cyclins E1 and E2) are found in mammals. Cyclins E1 and E2 are closely related and display very similar cell cycle-regulated expression and biochemical properties, in particular activation of CDK2 and inhibition by p27 (Gudas et al., 1999). In one study, cyclin E expression was found in syncytiotrophoblast and villous cytotrophoblasts and Western blot analysis revealed that both forms of cyclin E protein were present in the human placenta (DeLoia et al., 1997). In contrast, other studies reported that cyclin E was strongly expressed in villous cytotrophoblasts and some cells in the mesenchymal villous core but not in the terminally differentiated, non-dividing syncytiotrophoblast. In addition, extravillous cytotrophoblasts (EVTs) were often positive for cyclin E protein reaction (Ichikawa et al., 1998; McKenzie et al., 1998; Bamberger et al., 1999; Olvera et al., 2001). Cyclin E expression was downregulated in the differentiation of cultured cytotrophoblasts to syncytiotrophoblast (McKenzie et al., 1998). Moreover, cyclin E was strongly expressed in first trimester placental samples and decreased gradually toward term (Bamberger et al., 1999).

Cyclins A and B were expressed in villous cytotrophoblast and column trophoblasts but not in decidual cells and syncytiotrophoblast of the first trimester human placenta (Ichikawa et al., 1998; Korgun et al., 2006). Moreover, in the EVTs both of them were positive in one study (Korgun et al., 2006) while cyclin A was negative in another (Ichikawa et al., 1998). It is likely that differences in the sensitivity of techniques could account for the discrepancies between these two studies.

Cip/Kip family members (p21, p27 and p57)

CKIs are structurally and functionally divided into two groups: Cip/Kip and Ink4 families (Nakayama, 1998; Sherr and Roberts, 1999). p21 was shown to be localized to the syncytiotrophoblast and stromal cells, but not to the villous cytotrophoblasts, EVTs and decidual cells of first trimester human placenta (Korgun et al., 2006).

There are conflicting studies about the localization and density of p27 expression in human placentas. In some studies, p27 expression was heavily observed in syncytiotrophoblast, which is differentiated and does not divide, while villous cytotrophoblasts showed moderate staining in first trimester placentas (Bamberger et al., 1999; Olvera et al., 2001). However, in another study, in first trimester placentas villous cytotrophoblasts stained poorly and no p27 immune reactivity was detected in syncytiotrophoblast while at term, weak p27 expression was observed in syncytiotrophoblast (Genbacev et al., 2000). In another study, in first trimester placenta, p27 was immunolocalized to villous cytotrophoblasts, syncytiotrophoblast, stromal cells and decidual cells (Korgun et al., 2006). There are some studies showing increased p27 expression (Bamberger et al., 1999; Genbacev et al., 2000) while in another study p27

Table 1. Cyclins, CDK partners and their respective CKIs in cell cycle phases they are in charge of.

Cell cycle phase	Cyclin	CDK partner	CKI
G_1	Cyclin D	CDK4, CDK6	p15, p16, p18, p19, p21, p27, p57
G_1-S	Cyclin E	CDK2	p21, p27, p57
S	Cyclin A	CDK2	p21, p57
G_2-M	Cyclin B	CDK1	p21

staining decreased towards term (Olvera et al., 2001). p27 mRNAs were found to be upregulated in the terminal differentiation of cultured villous cytotrophoblasts to syncytiotrophoblast (McKenzie et al., 1998).

p57 expression has been identified in villous cytotrophoblasts, villous stromal cells, amniotic epithelium, invasive cytotrophoblasts and decidual cells of human placentas. However, it was not detected in syncytiotrophoblast and endometrial gland cells (Genbacev et al., 2000; Fukunaga, 2002; Korgun et al., 2006; Korkmaz et al., 2013). p57 was expressed mostly in invasive cytotrophoblasts and continued its positive immunoreactivity till term (Genbacev et al., 2000).

Ink4 family member p16

To the best of our knowledge, within the Ink4 family members only p16 has been studied in the placenta. p16 was implied in cytotrophoblast differentiation in the invasion of trophoblasts. In first trimester human placenta, p16 was confined to a few villous trophoblasts in the distal region of cell columns (Genbacev et al., 2000). p16 protein shows a gradual expression in cytotrophoblast, from p16 weak proliferative phenotype to a p16 strong invasive phenotype reaching a maximum around 17 weeks of human gestation (Candelier et al., 2013). In second trimester, staining of p16 significantly upregulated in association with syncytiotrophoblast, villous cytotrophoblasts and column cytotrophoblasts. Most cytotrophoblasts in all areas of the uterine wall stained for p16 (Genbacev et al., 2000).

p53

Tumor suppressor protein p53 protects cells from DNA damage by triggering apoptosis or by arresting the cell cycle to repair the damaged DNA. p53 along with p21 inhibits the progression of the cell cycle from G1 to S phase (Table 2). The stabilization of p53 upregulates p21, which inactivates CDK1 and cyclin B1. In the first trimester human placenta, p53 expression was at a normal level and present in villous cytotrophoblasts, syncytiotrophoblast and extravillous trophoblasts found in the cell column (Haidacher et al., 1995; Quenby et al., 1998; Korkmaz et al., 2013). Very few villous stromal cells were p53 positive. In term human placentas, p53 immunolabeling was very low and was expressed in only a limited number of syncytiotrophoblast cells (Korkmaz et al., 2013).

Proliferating cell nuclear antigen (PCNA)

One of the proteins synthesized in late G1 and S phases of the cell cycle is PCNA (Takahashi and Caviness, 1993) (Table 2). PCNA is a homotrimer protein that binds to a variety of factors required for cell cycle progression, replication and repair. Consequently, it plays important roles in DNA synthesis, repair and cell cycle regulation and is a commonly used proliferation marker (Start et al., 1992). PCNA relays both positive and negative signals in the cell cycle. It is a regulator of p21 activity by binding to the carboxyl terminus of p21, which results in the inhibition of PCNA-dependent DNA replication. Besides, p21 controls PCNA levels and when p21 is upregulated during differentiation, PCNA is downregulated (Maga and Hubscher, 2003). PCNA, in addition to being involved in the cell cycle has a role in DNA repair. Therefore, its expression might occur in DNA damaged cells as well as in proliferating ones (Jaskulski et al., 1988; Scott et al., 2001). As a result, immunohistochemical detection of PCNA in a cell shows that the cell is in cycle or its damaged DNA is being repaired (Andre et al., 2000). In the human placenta, the most intense expression of PCNA has been identified in villous and extravillous cytotrophoblasts. Moreover, PCNA was also expressed in syncytiotrophoblast, endothelial cells, villous stromal cells, decidual cells and decidual gland cells (Korgun et al., 2006; Korkmaz et al., 2013; Ünek et al., 2014b). PCNA expression was observed heavily in first trimester and reduced gradually towards term (Smith et al., 1998; Maruo et al., 2001; Danihel et al., 2002; Korkmaz et al., 2013).

Ki67

Another proliferation marker Ki67 is expressed during late G_1, S, G_2 and M phase of the cell cycle, while resting (G_0 phase) cells lack it (Table 2). Its role within the regulation of cell cycle is yet unknown (Gerdes et al., 1984). Ki67 shows a good correlation with the number of mitotic cells (Scholzen and Gerdes, 2000). Ki67 expression in human first trimester placentas was observed in villous cytotrophoblasts, villous stromal cells, invasive cytotrophoblasts and decidual cells. It was not detected in non-dividing syncytiotrophoblast and endometrial gland cells (Korgun et al., 2006). In many studies, Ki67 expression was intense in first trimester placental villous cytotrophoblasts (Ichikawa et al., 1998; Bamberger et al., 1999; Genbacev et al., 2000; Olvera et al., 2001; Xue et al., 2003; De Falco et al., 2004). In parallel with the expression of PCNA, Ki67 expression was highest in first trimester and reduced gradually over term (Smith et

Table 2. Some cell cycle related proteins and their respective cell cycle phases.

Cell cycle related protein	Cell cycle phase
PCNA	G_1, S
Ki67	Late G_1, S, G_2, M
p53	G_1, S

al., 1998; Bamberger et al., 1999; Genbacev et al., 2000; Olvera et al., 2001; Danihel et al., 2002; De Falco et al., 2004). Ki67 immunopositive reactivity was not observed in syncytiotrophoblast and EVTs of term placentas (Jeschke et al., 2006).

Immunohistochemical distribution of cell cycle activator (Fig. 1) and inhibitor (Fig. 2) proteins in human term placenta are illustrated. Immunohistochemical localizations of cell cycle related proteins in human placentas are reviewed in Table 3.

Table 3. Immunohistochemical localizations of cell cycle proteins in human placentas.

Cell cycle protein	Placental localization
PCNA	vCTs, EVTs, SynT, endothelial cells, villous stromal cells, decidual cells and decidual gland cells (Korgun et al., 2006; Korkmaz et al., 2013; Unek et al., 2014b)
Ki67	vCTs, villous stromal cells, decidual cells. Not detected in SynT, EVTs and endometrial gland cells (Jeschke et al., 2006; Korgun et al., 2006)
Cyclin A	vCTs and column trophoblasts but not in decidual cells and SynT of the first trimester human placenta (Ichikawa et al., 1998; Korgun et al., 2006); EVTs (Korgun et al., 2006); negative (Ichikawa et al., 1998)
Cyclin B	vCTs and column trophoblasts but not in decidual cells and SynT of the first trimester human placenta (Ichikawa et al., 1998; Korgun et al., 2006); EVTs (Korgun et al., 2006)
Cyclin D (D1, D2, D3)	Cyclin D1: intravillous vessel endothelial cells; Cyclin D2: villous core Cyclin D3: vCTs but was not expressed in SynT and EVTs (DeLoia et al., 1997; Genbacev et al., 2000)
Cyclin E	SynT and vCTs (DeLoia et al., 1997); vCTs, EVTs and mesenchymal villous core but not in the SynT (Ichikawa et al., 1998; McKenzie et al., 1998; Bamberger et al., 1999; Olvera et al., 2001)
p16	In first trimester, vCTs in the distal region of cell columns. In second trimester, in the SynT, vCTs and column cytotrophoblasts in both the proximal and the distal regions (Genbacev et al., 2000)
p21	SynT and stromal cells, but not in vCTs, EVTs and decidual cells (Korgun et al., 2006)
p27	Moderate staining in SynT, vCTs (Bamberger et al., 1999; Olvera et al., 2001); Weak staining of vCTs and SynT (Genbacev et al., 2000); vCTs, SynT and stromal cells and decidual cells (Korgun et al., 2006)
p57	vCTs, villous stromal cells, amniotic epithelium, EVTs and decidual cells. It was not detected in SynT and endometrial gland cells (Genbacev et al., 2000; Fukunaga, 2002; Korgun et al., 2006; Korkmaz et al., 2013)
p53	vCTs, SynT and EVTs found in the cell column, very few villous stroma cells (Haidacher et al., 1995; Quenby et al., 1998; Korkmaz et al., 2013)

Villous cytotrophoblasts: vCTs, extravillous cytotrophoblasts: EVTs, syncytiotrophoblast: SynT.

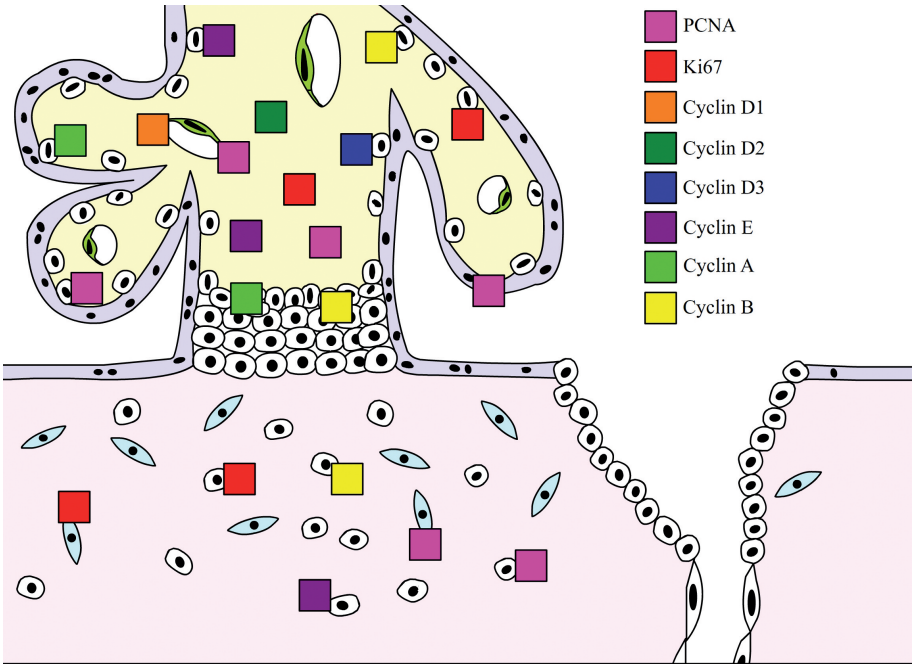

Fig. 1. Immunohistochemical distribution of cell cycle activator proteins in human term placenta.

Placental morphological and cell cycle related protein expression changes in pregnancy pathologies

Preeclampsia

Preeclampsia (PE) is a hypertensive disease, which is clinically identified as a pregnancy-induced high blood pressure of more than 140/90 with proteinuria of greater than 300 mg protein in a 24 h period. It is one of the leading causes of maternal and fetal morbidity and mortality in developing countries (Papageorghiou et al., 2001). Preeclamptic placentas are smaller than normal and the placenta/fetal weight ratio is known to decrease (Benirschke et al., 2006).

Preeclamptic placental trophoblast cells are known to have abnormal cell cycle mechanisms and increased apoptosis (Heazell et al., 2008). There is a study suggesting that the distribution of Ki67 and p53 proteins in preeclamptic placentas is similar to normal placentas (Jeschke et al., 2006). In addition, in another study, Ki67, cyclin A and cyclin B expression did not change between normal and preeclamptic villous cytotrophoblasts (DiFederico et al., 1999). However, in a study with PE placental explants, excessive apoptosis and lack of proliferation stimulation (Ki67 expression) were observed in the trophoblast cells (Crocker et al., 2004). Moreover, in other studies, PCNA and Ki67 immunoreactivities increased in villous cytotrophoblasts of preeclamptic placentas (Jones and Fox, 1980; Hustin et al., 1984; Arnholdt et al., 1991; Redline and Patterson, 1995; Lyall and Myatt, 2002; Unek et al., 2014a), which indicated an increased proliferative activity compared to normal. Syncytiotrophoblast which was damaged by hypoxia resulting from ischemia is thought to be regenerated by increased proliferation in villous cytotrophoblasts (Arnholdt et al., 1991). Numerous morphometric data show that the number of villous cytotrophoblasts is higher in PE than normal pregnancies (Soma et al., 1982; Teasdale, 1985; DiFederico et al., 1999).

Increased villous cytotrophoblast apoptosis was reported in PE compared to normal pregnancies (Allaire et al., 2000; Levy et al., 2002). In addition, in term placentas, syncytiotrophoblast apoptosis was higher in PE than normal placentas (Ishihara et al., 2002). In our study, immunostaining of cell cycle inhibitor p27 increased in villous cytotrophoblasts and syncytiotrophoblast of preeclamptic placentas (Unek et al., 2014a). Because there is a positive correlation between cell cycle inhibitor expression and apoptosis (Katayose et al., 1997; Samuelsson et al., 2002; Wu et al., 2002; Vlachos et al., 2007; Yoshida et al., 2012), our findings were consistent with the finding that apoptosis increased in cytotrophoblasts and syncytiotrophoblast of preeclamptic placentas compared to that of normal. Based on these findings, a theory in which villous cytotrophoblast turnover involving the proliferation, fusion and apoptosis increased in PE compared to normal was put forward (Huppertz and Kingdom, 2004).

It was shown by TUNEL method that 15%-50% of EVTs underwent apoptosis in PE. This increased apoptosis is thought to be responsible for the reduced EVT invasion in PE (DiFederico et al., 1999). Abruptio placenta, early separation of placenta from the uterus because of inadequate invasion to decidua, was seen in

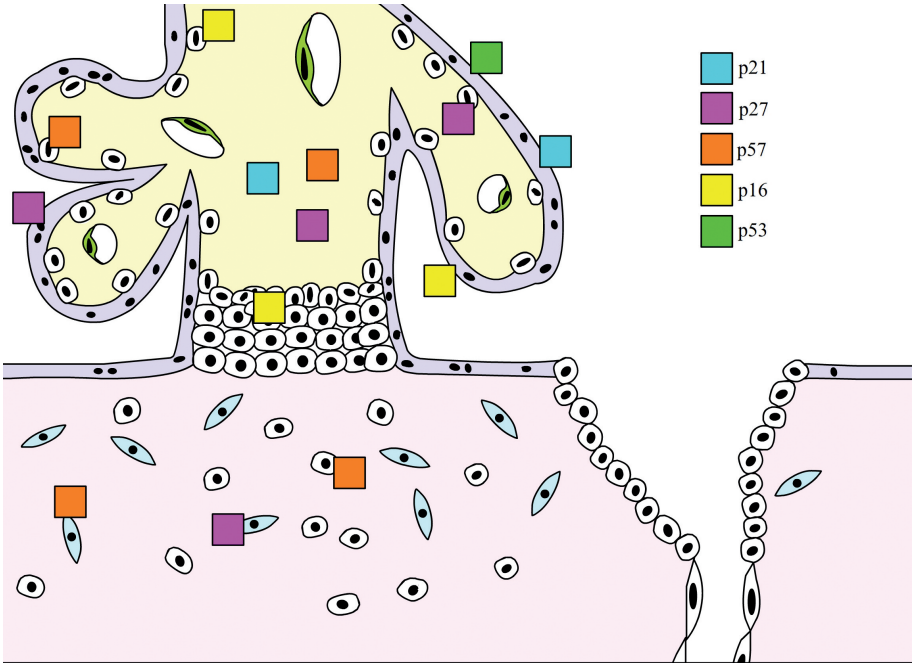

Fig. 2. Immunohistochemical distribution of cell cycle inhibitors in human term placenta.

PE three times more frequently than normal (Bulmer, 1992). This may be due to increased apoptosis of EVTs. In accordance, immunostainings of proliferative markers (PCNA and Ki67) decreased significantly in basal plates (particularly in EVTs) of preeclamptic placentas. Moreover, EVT stainings of cell cycle inhibitors (p27 and p57) greatly increased in preeclamptic placentas (Unek et al., 2014a).

In preeclamptic chorionic plates immune stainings of PCNA and Ki67 increased significantly in chorionic cytotrophoblasts (Unek et al., 2014a). Increased proliferation in chorionic cytotrophoblasts of PE might be due to the increased cytotrophoblast turnover. Furthermore, cyclin D1 expression was significantly downregulated in PE relative to control in cultured placental mesenchymal stromal cells (Nuzzo et al., 2014). In parallel, immunoreactivities of p27 and p57 increased in amnion epithelium and chorionic stromal cells of preeclamptic placentas compared to control (Unek et al., 2014a).

Immunohistochemical expression changes of cell cycle related proteins in PE cases compared to normal placentas are summarized in Table 4.

Intrauterine Growth Restriction (IUGR)

The fetus which is below the standard weight for gestational age at the rate of 10% or more, is referred to have intrauterine growth restriction (IUGR). The incidence of IUGR in developed countries is 3%, while in developing countries it is over 10% (Creasy and Resnik, 1994). Malnutrition, fetal infections (5%), chromosomal abnormalities such as trisomy 21, bone and cartilage disorders, teratogens, vascular diseases such as preeclampsia and hypertension, renal diseases, living at high altitude, anemia, placental and umbilical cord abnormalities and risk of multiple pregnancies are risk factors for IUGR. Non-pathological risk factors of IUGR are the height and weight of parents, pregnancy in old age, ethnicity and fetal gender (Seeds, 1984).

The pathogenesis of IUGR can be divided into two groups as placental hyper- and hypooxygenation. These groups differ in terms of trophoblastic proliferative activity (Blankenship and King, 1994; Chan et al., 1999). In placental hyperoxygenation, there is decreased proliferative activity, while in placental hypo-oxygenation, trophoblastic proliferative activity increases depending on the decreased maternal blood flow. In 84% of cases of IUGR, proliferative index is low or medium level (Garnica and Chan, 1996).

IUGR placentas are significantly smaller and lighter than the normal placentas (Biswas and Ghosh, 2008). IUGR placentas showed reduced expression of proliferative markers PCNA, Ki67 and cyclin D3 in villi, especially in villous cytotrophoblasts (Garnica and Chan, 1996; Unek et al., 2014a,b). In another study, in IUGR cytotrophoblasts no statistically significant change in Ki67 expression but a statistically significant downregulation of p53 was found (Jeschke et al., 2006). Moreover, in chorionic plates and basal plates of IUGR placentas, proliferation decreased in all cell types (Unek et al., 2014a,b). Apoptosis in IUGR placentas has been shown to be higher than usual, especially in villous cytotrophoblasts and stromal tissue (Axt et al., 1999; Erel et al., 2001; Ishihara et al., 2002; Levy et al., 2002; Endo et al., 2005). Accordingly, the expression of cell cycle inhibitors p16, p21, p27 and p57 was significantly elevated in IUGR placental samples especially in villi and chorionic plates (Davy et al., 2009; Unek et al., 2014b).

The number of syncytiotrophoblast nuclei subjected to apoptosis was also higher than normal in IUGR term placentas (Ishihara et al., 2002). In addition, increased apoptosis was observed in the trophoblasts of chorionic mesenchyme of IUGR placentas (Murthi et al., 2005).

Immunohistochemical expression changes of cell cycle related proteins in IUGR placentas compared to normal are shown in Table 5.

Table 4. Immunohistochemical expression changes of cell cycle proteins in PE cases compared to normal placentas.

Cell cycle protein	Expression changes in PE placentas compared to normal
PCNA	In vCTs ↑ (Redline and Patterson, 1995); In vCTs, chorionic cytotrophoblasts ↑ (Unek et al., 2014a) In EVTs ↓ (Unek et al., 2014a)
Ki67	In vCTs , villous stromal cells ↓(Crocker et al., 2004); In vCTs ↔ (DiFederico et al., 1999; Jeschke et al., 2006) In vCTs ↑ (Arnholdt et al., 1991); In vCTs , chorionic cytotrophoblasts ↑ (Unek et al., 2014a) In EVTs ↓ (Unek et al., 2014a)
Cyclin A	In vCTs ↔ (DiFederico et al., 1999)
Cyclin B	In vCTs ↔ (DiFederico et al., 1999)
Cyclin D1	In cultured placental mesenchymal stromal cells ↓ (Nuzzo et al., 2014)
p27	In vCTs, SynT, EVTs, amnion epithelium and chorionic stromal cells ↑ (Unek et al., 2014a)
p57	In EVTs, amnion epithelium and chorionic stromal cells ↑ (Unek et al., 2014a)
p53	↔ (Jeschke et al., 2006); In vCTs ↑(Levy et al., 2002)

Villous cytotrophoblasts: vCTs, extravillous cytotrophoblasts: EVTs, syncytiotrophoblast: SynT, ↔: Expression not changed, ↑: Increased expression, ↓: Decreased expression.

Diabetes mellitus

Diabetes Mellitus is a disease causing acute metabolic complications and long-term vascular, renal, retinal or neuropathic disorders, with high morbidity and mortality risks (Alemzadeh, 2004; Burant, 2004). Diabetic pregnancies are risky for both mother and fetus. Some of these risks include fetal macrosomia, spontaneous abortions, congenital malformations, stillbirth, polyhydramnios, maternal hypertension, ketoacidosis, diabetic retinopathy, neuropathy, nephropathy and preeclampsia (Kuhl, 1975; Pickup and Williams, 1997).

The diabetic environment may have profound effects on placental development and function. The most common abnormality of diabetic patients' placentas is villous immaturity. Villous immaturity is characterized by an increase in the number of mature and immature intermediate villi and a reduction in the number of terminal villi with respect to gestational age. 60% of the diabetic placentas have villous immaturity (Benirschke et al., 2006). Villous immaturity is thought to be an indicator of a problem in the vascular development (Charnock-Jones et al., 2004). Villous immaturity, by increasing the diffusion distance between the intervillous space and fetal capillaries, leads to chronic fetal hypoxia (Laurini et al., 1987).

Diabetic placentas of pregnant women are thick and plethoric because of maternal hyperglycemia and fetal hypervolemia (Padmanabhan and Shafiullah, 2001). Placental weight tends to be heavier in diabetes, similar to fetal weight, but the weight gain is more pronounced in the placenta than in the fetus, as is reflected in a higher placental-to-fetal weight ratio than in normal gestation. Placentomegaly is correlated with fetal macrosomia confirming the close correlation of placental weight with that of the offspring (Taricco et al., 2003). Placentomegaly is due to the increase in parenchymal tissue cells that is evidenced by high DNA content (Padmanabhan and Shafiullah, 2001). It is possible that this increased cell proliferation promotes thickness of the placental barrier consequently affecting the normal maternal-fetal exchanges.

Increased proliferative activity was reported in diabetic placentas' villous cytotrophoblasts compared to normal (Jones and Fox, 1976; Desoye and Shafrir, 1996). Moreover, in diabetic placentas villous cytotrophoblast number was higher than normal (Fox, 1969; Desoye and Shafrir, 1996). In DM patients' placentas, epidermal growth factor receptor expression, which induces the differentiation of cytotrophoblasts to syncytiotrophoblast, decreases. Therefore, villous

Table 5. Immunohistochemical expression changes of cell cycle related proteins in IUGR placentas compared to normal.

Cell cycle protein	Expression changes in IUGR placentas compared to normal
PCNA	↓ (Unek et al., 2014b)
Ki67	In vCTs ↔ (Jeschke et al., 2006); ↓ (Unek et al., 2014b)
Cyclin D3	In villi especially in vCTs ↓ (Unek et al., 2014b)
p16	↑ (Davy et al., 2009)
p21	↑ (Davy et al., 2009)
p27	In villi and chorionic plates ↑ (Unek et al., 2014b)
p57	In villi and chorionic plates ↑ (Unek et al., 2014b)
p53	In vCTs ↓ (Jeschke et al., 2006)

Villous cytotrophoblasts: vCTs, extravillous cytotrophoblasts: EVTs, syncytiotrophoblast: SynT, ↔: Expression not changed, ↑: Increased expression, ↓: Decreased expression.

Table 6. Immunohistochemical expression changes of cell cycle proteins in DM placentas compared to normal placentas.

Cell cycle protein	Expression changes in DM placentas compared to normal
PCNA	In placental endothelial cells ↑ (Leach et al., 2004); In vCTs, SynT, villous stromal cells and fetal endothelial cells ↑ (Unek et al., 2013)
Ki67	In villi and EVTs ↑ (Unek et al., 2013); In vCTs, SynT, villous stromal cells and fetal endothelial cells (Burleigh et al., 2004)
Cyclin D3	In villi and chorionic plates ↑ (Unek et al., 2013)
p27	In vCTs ↑ (Unek et al., 2013); In chorionic plates ↓ (Unek et al., 2013)
p57	In chorionic plates ↓ (Unek et al., 2013)

Villous cytotrophoblasts: vCTs, extravillous cytotrophoblasts: EVTs, syncytiotrophoblast: SynT, ↔: Expression not changed, ↑: Increased expression, ↓: Decreased expression.

cytotrophoblasts may proliferate rather than differentiate to syncytiotrophoblast (Desoye and Shafrir, 1996). A study showed that at 21% oxygen, hyperglycemia led to decreased cell proliferation compared to normoglycemia in a first trimester trophoblast-derived cell line. Cell cycle analysis showed a significant increase in cells in S phase and a tendency of decreased G_1 phase under hyperglycemia at 21% oxygen (Frohlich et al., 2012). In another study, expressions of genes involved in the cell cycle were upregulated under hyperglycemia compared with hypoglycemia conditions in human trophoblast BeWo cell line. In line with the previous study, G_2/M cell cycle progression was enhanced under hyperglycemia conditions. Consistent with this, cyclin E expression was markedly lower in hyperglycemia compared to the hypoglycemia conditions, suggesting that G_1/S transition was inhibited (Inadera et al., 2010).

In addition, in the villi of diabetic placentas increased proliferation of other cell types are observed. For example, the number of villous stromal fibroblasts, villous macrophages, endothelial cells and syncytiotrophoblast nucleus were found to be higher than normal (Teasdale, 1981; Desoye and Shafrir, 1996). PCNA immunoreactivity in endothelial cells of diabetic placentas was five times higher than normal (Leach et al., 2004). In our study, PCNA staining of villous cytotrophoblasts, syncytiotrophoblast, villous stromal cells and fetal endothelial cells showed an increase in diabetic placentas. Similarly, Ki67 and cyclin D3 staining of villous parts also increased in diabetic placentas (Unek et al., 2013). In another study, Ki67 expression in villous cytotrophoblasts, syncytiotrophoblast, villous stromal cells and fetal endothelial cells did not differ between normal and diabetic placentas. However, in diabetic placentas, apoptosis of villous stromal cells was shown to be less than normal (Burleigh et al., 2004). In addition, increased apoptosis of villous cytotrophoblasts and syncytiotrophoblast nuclei were observed in diabetic placentas compared to normal (Sgarbosa et al., 2006). In parallel, increased p27 staining was found in villous cytotrophoblasts of diabetic placentas. In addition to the villous parts, Ki67 and cyclin D3 stainings also significantly increased in EVTs and chorionic plates of diabetic placentas respectively. In chorionic plates, cell cycle inhibitors p27 and p57 staining intensities significantly decreased in diabetic group (Unek et al., 2013).

Immunohistochemical expression changes of cell cycle proteins in diabetic placentas compared to normal placentas are summarized in Table 6.

Conclusions

The placenta critically influences the outcome of the pregnancy and fetal growth is directly linked to placental development. Placental development relies on the coordinated action of trophoblasts' proliferation, differentiation and invasion that are controlled by cell cycle regulator proteins. Placental development is compromised in pregnancy pathologies such as preeclampsia, intrauterine growth restriction, diabetes mellitus and gestational trophoblastic diseases. In this review, we attempted to link cell cycle regulator proteins with placenta physiology and presented evidence suggesting that failure to develop a normal placenta could be the result of abnormal cell cycle regulation. It is reviewed here that there are numerous changes in the expression and spatio-temporal localizations of cell cycle related proteins between normal and pathological placentas. Some of the morphological changes observed in pathological placentas might be due to the differences in the expression of cell cycle proteins between normal and pathological placentas. Placental dysfunction of pathological placentas might be linked to proliferation and cell cycle arrest mechanisms' alterations occurring in the pregnancy pathologies. In order to understand pregnancy pathologies, develop effective and accurate approaches to predict and eventually produce treatments, placental development requires to be delineated.

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