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The expression of cell cycle related proteins PCNA, Ki67, p27 and p57 in normal and preeclamptic human placentas

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ABSTRACT

Placenta is a transitional area making many physiological activities between mother and fetus and therefore, it is a critical organ influencing the outcome of pregnancy. Fetal growth is directly related to placental development. Accurate placental development depends on coordinated action of trophoblasts' proliferation, differentiation and invasion. Information on cell cycle related proteins that control these events is limited and how they are affected in preeclampsia is not fully understood yet. Therefore, in this study, in order to understand the role of cell cycle regulators in preeclamptic placentas we aimed to determine the spatio-temporal immunolocalizations of cell cycle regulators in preeclamptic and normal human term placentas. Term placentas were obtained from women diagnosed with preeclampsia and from normal pregnancies with informed consent following cesarean deliveries. Placental samples were stained via immunohistochemistry with PCNA, Ki67, p27, p57, vimentin and cytokeratin 7 antibodies and were examined by light microscopy. PCNA and Ki67 staining intensities significantly increased in villous parts, significantly decreased in basal plates of PE group and did not change in chorionic plates. Staining intensities of cell cycle inhibitors p27 and p57 significantly increased in all parts of preeclamptic placentas compared to control. Placental abnormalities of preeclamptic placentas might be associated with proliferation and cell cycle arrest mechanisms' alterations occurred in preeclampsia.

1. Introduction

Placenta which is a transitional zone regulating many metabolic activities between mother and fetus is a critical organ affecting the outcome of pregnancy. Proper placental development is required for a healthy fetus. Abnormal placental development constitutes one of the biggest causes of early embryonic death. Normal placental development depends on the cell proliferation, differentiation and invasion in a proper and simultaneous manner (Genbacev et al., 2000).

Eukaryotic cell cycle is regulated by cyclins and cyclin-dependent kinases (CDKs) in a coordinated manner, and controlled by cyclin dependent kinase inhibitors (CKIs) (De Falco et al., 2004).

CKIs by binding to cyclins, CDKs or cyclin-CDK complexes inhibit the activities of CDKs. CKIs are structurally and functionally divided into two groups: Ink4 and Cip/Kip families (Nakayama, 1998; Sherr and Roberts, 1999). Cip/Kip family members, p21, p27 and p57, by binding to cyclin D, -E, -A or -B/CDK complexes inhibit the activities of them (Sherr and Roberts, 1999; Zieske, 2000). p27 interacts with D-type cyclins/CDK4 complexes strongly and with cyclin E/CDK2 complex weakly and is responsible for G1 phase arrest (Pollard and Earnshaw, 2002; Weinberg, 1991). p57 inhibits cyclin D/CDK4, cyclin A/CDK2 or cyclin E/CDK2 complexes and thereby blocks G1/S transition and completion of S phase (Matsuoka et al., 1995).

There are conflicting studies about the localization and density of p27 expression in normal 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 has been detected in syncytiotrophoblast and at term, weak expression was observed in syncytiotrophoblast (Genbacev et al., 2000). There are some studies showing increased p27 expression (Bamberger et al., 1999; Genbacev et al., 2000) while in another study p27 staining decreased (Olvera et al.,

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Table 1
Clinical characteristics of the study groups.

	PE $n = 10$	Control $n = 10$	p-Value
Maternal age (years)	30.0 ± 4.082	27.40 ± 4.92	0.215
Gestational age (weeks)	35.5 ± 3.48	37 ± 0.82	0.1
Fetal birth weight (kg)	2.39 ± 0.98	2.93 ± 0.28	0.09
Placental weight (g)	351.80 ± 81.95	462.00 ± 55.95	<0.05*
Placenta/fetus weight ratio	0.14 ± 0.016	0.17 ± 0.013	<0.05*
Systolic blood pressure (mmHg)	153.2 ± 10.80	111.23 ± 6.23	<0.001*
Diastolic blood pressure (mmHg)	102.4 ± 8.75	76.02 ± 9.09	<0.001*
Proteinuria (g/24 h)	0.48 ± 0.12	–	–

Data are shown as mean ± S.D.
* Values significantly different from control group ($p < 0.05$; Student's t-test).

2001) toward term. 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 (Fukunaga, 2002; Genbacev et al., 2000; Korgun et al., 2006). p57 was expressed mostly in invasive cytotrophoblasts and continued its positive immunoreactivity till term (Genbacev et al., 2000).

One of the proteins synthesized in late G1 and S phases of the cell cycle is PCNA (Takahashi and Caviness, 1993). PCNA plays important roles in DNA synthesis, repair and cell cycle regulation and a commonly used proliferation marker (Start et al., 1992). In the human placenta, the most intense expression of PCNA has been identified in villous and invasive cytotrophoblasts. Moreover, PCNA was also expressed in syncytiotrophoblast, villous stromal cells, decidual cells and decidual gland cells (Korgun et al., 2006). PCNA expression was observed heavily in first trimester and was reduced gradually toward term (Danihel et al., 2002; Maruo et al., 2001; Smith et al., 1998).

Another proliferation marker Ki67 is expressed during late G1, S, G2 and M phase of the cell cycle, while resting (G0 phase) cells lack it (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 syncytiotrophoblast and endometrial gland cells (Korgun et al., 2006). Ki67 immunopositive reactivity was not observed in syncytiotrophoblast and extravillous trophoblasts (EVT) of term placentas (Jeschke et al., 2006).

Preeclampsia (PE) is one of the leading causes of maternal and fetal morbidity and mortality in developing countries (Papageorghiou et al., 2001). The etiology of PE is not completely understood (Roberts et al., 1989). Physiological changes in spiral arteries is impaired in PE which ultimately leads to placental hypoxia and reduction in the blood supply to the fetus (Papageorghiou et al., 2001; Salafia and Shiverick, 1999). Preeclamptic placentas are smaller than normal and the placenta/fetal weight ratio is known to decrease. Placental infarcts because of inadequate intervillous circulation are one of the most observed placental changes in preeeclampsia (Benirschke et al., 2006).

Preeclamptic placental trophoblast cells are known to have abnormal cell cycle mechanisms and increased apoptosis (Heazell et al., 2008). There are hypotheses suggesting distribution of Ki67 protein in preeclamptic placentas is similar to normal placentas available (Jeschke et al., 2006). However, in a study with PE placental explants, excessive apoptosis and lack of proliferation stimulation were observed in the trophoblast cells (Crocker et al., 2004).

Although placental growth is mainly due to the coordination of trophoblast proliferation and differentiation, there is little information about the mitotic regulators that provide the synchronization of trophoblast proliferation and differentiation (Levy et al., 2002). The information on how trophoblast proliferation and differentiation are coordinated and what factors provide trophoblast cells to divide or differentiate is limited (Korgun et al., 2006). Preeclamptic placentas do not undergo normal development. How PE affects the coordination of trophoblast proliferation and differentiation is unknown. Therefore, to understand the role of cell cycle regulators in pathological human term placentas will be extremely useful in this regard. In this study, it is aimed to determine the spatio-temporal immunolocalizations of cell cycle regulators PCNA, Ki67, p27 and p57 in preeclamptic human term placentas.

2. Materials and methods

2.1. Tissue samples

Term placentas from healthy women were used as control group ($n = 10$). Placentas from women diagnosed with PE ($n = 10$) and control group placentas were obtained immediately after cesarean deliveries. Immediately after birth, placentas were brought to the laboratory on dry ice. Tissues were supplied from the Department of Obstetrics and Gynecology, Faculty of Medicine, Antalya. Informed consent was obtained from the patients. The study was approved by the Ethical Committee of the Faculty of Medicine in Akdeniz University.

Preeclampsia was defined by increased blood pressure (≥140 mmHg systolic and ≥90 mmHg diastolic on ≥2 occasions at least 6 h apart) that occurred after 20 weeks of gestation in a woman with previously normal blood pressure, accompanied by proteinuria (≥0.3 g/24 h). Clinical data from the two groups are given in Table 1.

2.2. Tissue processing

The placentas were dissected, washed with phosphate-buffered saline (PBS, pH 7.4) a few times to take blood away from tissues and fixed in Holland's fixative (consisting of 4% formaldehyde, 5 ml glacial acetic acid, 4 g picric acid and 2.5 g cupric acetate in 100 ml distilled water) at room temperature for 6 h for immunohistochemical studies. Then, tissues were washed in tap water for 6–7 h and dehydrated with 70%, 80% and 90% ethanol series for one day each respectively. After tissues were kept in 100% ethanol for 3 hours, dehydration step finished. Then, tissues were cleared in xylene 3 times for 3 minutes each, paraffinized 3 times for 1 h each and finally embedded in paraffin.

2.3. Routine light microscopy observations

5 μm thick sections were obtained from paraffin blocks of placental samples. Hematoxylin-eosin stainings of sections were examined by light microscopy (Zeiss Axioplan, Germany) and histopathologic findings were recorded. The sampling sites were