Upsala J Med Sci 113 (2): 235–242, 2008

Comparison of Placental PTEN and β1 Integrin Expression in Early Spontaneous Abortion, Early and Late Normal Pregnancy

Cigdem Tokyol[1], Fatma Aktepe[1], Fatma Hüsniye Dilek[1], Mehmet Yilmazer[2]

Departments of [1]Pathology, and [2] Gynecology and Obstetrics, Afyon Kocatepe University School of Medicine, Afyonkarahisar, Turkey

Abstract

Background: PTEN seems to play an important role in cell cycle, growth, migration, and death. Integrins are cell surface receptors that play a role in the regulation of cell proliferation, differentiation, implantation, and embryogenesis. PTEN inhibits β1 integrin signaling. The objective of this study is to investigate the expression of PTEN and β1 integrin in placental tissues of early spontaneous abortion and first and third trimesters of normal pregnancy.

Method: A total of 43 placental tissue samples were evaluated using immunohistochemistry for PTEN and β1 integrin. Group 1 included placental tissues of volunteer termination of normal pregnancy during the first trimester (5–10 wk gestation). Group 2 included placental tissues of normal vaginal delivery at the third trimester of pregnancy (36–40 wk gestation). Group 3 included placental tissues of pregnancy termination because of spontaneous abortion during the first trimester (5–10 wk gestation).

Results: PTEN expression of villous trophoblast was decreasing as the pregnancy advanced. PTEN staining of decidual cells was significantly stronger in tissue samples from early spontaneous abortion than in tissue samples from early and late normal pregnancy (p=0.003, p=0.001, respectively). There was no significant difference between β1 integrin expression of villous trophoblast and decidual cells in three groups.

Conclusion: Our findings suggest that altered patterns of PTEN expression may be associated with abortion, but it seems that β1 integrin does not contribute to this process as a signaling protein. Further evaluation is needed to highlight this subject.

Introduction

PTEN (*phosphatase and tensin homolog deleted on chromosome* 10) is a tumor suppressor gene which was identified in 1997 (1–3). The PTEN gene is frequently deleted or mutated not only in prostatic, endometrial, breast, lung, kidney, bladder, testis, head and neck cancers, but also in glioblastoma, malignant melanoma, and lymphoma (4).

PTEN seems to play an important role in cell cycle, growth, migration, and death (5). The PTEN gene encodes a dual-specifity protein phosphatase and also has extensive homology to tensin, a protein that interacts with actin filaments at focal adhesions. Focal adhesions are sites on the plasma membrane at which in-

Key words: PTEN; β1 integrin; immunohistochemistry; placenta; abortion

Received 9 January 2008
Accepted 14 April 2008

tegrins aggregate (6). Integrins are transmembrane glycoproteins made up of α and β chains (7). β1 integrins interact with a number of signal transduction proteins, including focal adhesion kinase as well as cytoskeletal proteins. In this manner integrins mediate processes such as cell migration, spreading, and growth (4,6). PTEN inhibits the phosphorylation of focal adhesion kinase in response to integrin-mediated processes (6).

Integrins are cell surface receptors that play a role in the regulation of cell proliferation, differentiation, implantation, and embryogenesis (8,9). PTEN inhibits β1 integrin signaling. A variety of normal cells undergo apoptosis when they lose attachment to an appropriate extracellular matrix. Thus, PTEN induces apoptosis (4).

Endometrial functions are carried out by mechanisms of proliferation, differentiation, implantation, and apoptosis. It has been shown that endometrial and decidual integrin and PTEN expression change throughout the menstrual cycle and pregnancy (8,10–14). Integrins and PTEN are also expressed in normal human placental tissue (15,16).

The objective of this study is to investigate the expression of PTEN and β1 integrin in placental tissues of early spontaneous abortion and first and third trimesters of normal pregnancy and evaluating a comparison between them. Such markers may be useful in promoting our understanding of a common pathway for spontaneous abortion regardless of the etiology.

Methods

We performed a retrospective study including three separate series of paraffin-embedded placental tissue samples collected from the pathology files of our hospital from 2001 to 2004.

The first series of samples (Group 1) included placental tissues of 15 women who underwent volunteer termination of clinically normal pregnancy during the first trimester (5–10 wk gestation).

The second series of samples (Group 2) included placental tissues of 15 women who had normal vaginal delivery at the third trimester of pregnancy (36-40 wk gestation).

The third series of samples (Group 3) included placental tissues of 13 women who underwent pregnancy termination because of spontaneous abortion during the first trimester (5–10 wk gestation). Gestational ages were calculated using the last menstrual period. All spontaneous abortion patients had ultrasonographic evaluation when they presented with vaginal bleeding and uterine evacuation was performed after ultrasonographic evaluation. Women with serious systemic disease (diabetes mellitus, thyroid dysfunction, infectious disease… etc.) and anembryonic pregnancies were not included in the study.

Immunohistochemistry

The streptavidin-biotin-peroxidase method was performed using the primary monoclonal antibodies against PTEN Ab-4 (Clone 17.A, prediluted, Neomarkers, USA) and CD29 (İntegrin Beta-1) Ab-3 (Clone 29CO3, prediluted, Neomarkers, USA). The representative blocks of placental tissues were sectioned and mounted on poly-L-lysin-coated slides. Before immunohistochemistry epitope retrieval was performed by boiling the slides in 10 mM citrate buffer, pH 6.0, for 20 min in a microwave oven. Slides were cooled at room temperature for 20 min. Endogenous peroxidase activity was blocked using hydrogen peroxide for 10 min. Tissues were incubated with blocking serum for 5 min to avoid nonspecific background staining and washed in tris buffered saline (TBS). Primary monoclonal antibody against PTEN and β1 integrin was then applied for 30 min and 1 h respectively at room temperature and washed in TBS. Linking antibody and streptavidin peroxidase (Lab Vision) were added consecutively for 10 min and washed in TBS. The peroxidase activity was visualized with 3-Amino-9-Ethylcarbazole for 7 min. The slides were counterstained in Mayer' s hematoxylin. Positive staining for β1 integrin was defined as immunoreactivity at the cell membrane and for PTEN cytoplasmic staining. Positive controls consisted of known positive samples of placenta.

PTEN and β1 integrin expression were evaluated blindly in villous trophoblast and decidual cells. Decidual cells were distinguished from intermediate-type trophoblast by the lack of significant nuclear atypia. The degree of positive staining for PTEN was evaluated using a semiquantitative scale which was described by Taniyama et al (17): 1) Negative 2) ≤5% immunoreactive trophoblastic/decidual cells 3) 5-50% immunoreactive trophoblastic/decidual cells 4) ≥50% immunoreactive trophoblastic/decidual cells. The degree of positive staining for β1 integrin was evaluated using a semiquantitative scale which was described by Manzotti et al (18): 1) Negative 2) ≤10% immunoreactive trophoblastic/decidual cells 3) 10–50% immunoreactive trophoblastic/decidual cells 4) ≥50% immunoreactive trophoblastic/decidual cells.

Statistical analysis

The immunohistochemical data are reported as the mean ± standard error of mean (SEM). Statistical analysis of the data was performed using Kruskal-Wallis and Mann-Whitney *U* tests. Bivariate correlation between variables was determined by Pearson' s correlation coefficients. A p value <0.05 was considered significant.

Results

Results of the immunohistochemical staining have been summarized in Table 1.

The staining pattern of PTEN was cytoplasmic. Expression of PTEN was prominent in villous trophoblasts in early spontaneous abortion and early pregnancy

Table 1. PTEN and β1 integrin immunoreactivity in placental tissues

Tissues	PTEN		β1 INTEGRIN	
	Trophoblast	Decidua	Trophoblast	Decidua
Normal pregnancy at first trimester (n=15)	3.1(0.3)	1.1(0.1)	2.0(0.2)	2.5(0.2)
Normal pregnancy at third trimester (n=15)	1.0(0.0)*	1.0(0.0)	2.8(0.3)	2.4(0.3)
Spontaneous abortion at first trimester (n=13)	2.6(0.3)	2.2(0.3)**	2.1(0.3)	2.6(0.2)

SEM is reported in *parentheses*.
*p<0.01 for Group 2 vs Group 1 and Group 3.
**p<0.01 for Group 3 vs Group1 and Group 2.

(Fig.1). There was no PTEN staining in villous trophoblasts and decidual cells in late pregnancy. PTEN staining of villous trophoblasts was significantly stronger in tissue samples from early pregnancy and early spontaneous abortion than samples from late pregnancy (p=0.000, p=0.000). PTEN expression of villous trophoblast was decreasing as the pregnancy advanced. Though there was very weak PTEN staining in decidual cells in early pregnancy, expression of PTEN was prominent in decidua in early spontaneous abortion (Fig.2). PTEN staining of decidual cells was significantly stronger in tissue samples from early spontaneous abortion than in tissue samples from early and late pregnancy (p=0.003, p=0.001, respectively).

Staining for β1 integrin revealed positivity around cell membranes in villous trophoblasts (Fig.3), and decidual cells (Fig.4). Although we have observed β1 integrin expression in villous trophoblasts and decidual cells in three groups, there was no statistically significant difference between them.

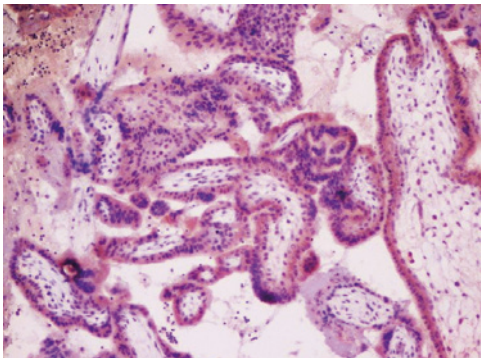

Figure 1. Cytoplasmic staining of PTEN in villous trophoblasts in spontaneous abortion (x200).

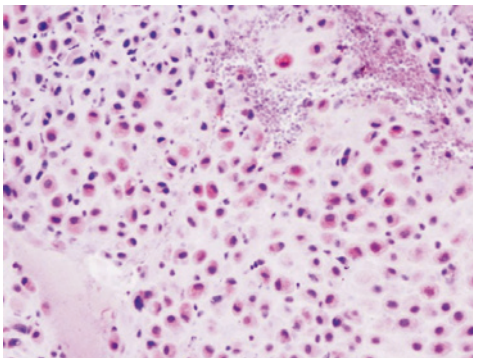

Figure 2. Cytoplasmic staining of PTEN in decidual cells in spontaneous abortion (x200).

Discussion

PTEN has a critical importance during development and embryogenesis. It has been shown that mice with homozygous-targeted deletion of PTEN gene have abnormal patterning of ectodermal and mesodermal germ layers and defective placentation (19,20). PTEN is expressed in placental tissue and is essential for embryonic development (21).

In a previous study, PTEN expression was evaluated throughout the menstrual cycle in normal endometrial tissues. It was reported that proliferative endometrium showed cytoplasmic and nuclear PTEN expression in the surface epithelium. By the midsecretory phase, epithelial PTEN is exhausted, but increases dramatically in the cytoplasm of stromal cells undergoing decidual change. It was concluded that stromal and epithelial compartments contribute to the hormone-driven changes in endometrial PTEN expression and inferred that abnormal hormonal conditions may disrupt normal patterns of PTEN expression in this tissue (13).

Kayışlı et al evaluated PTEN expression throughout the menstrual cycle and during early pregnancy. They found higher PTEN immunoreactivity in endometrial stromal and glandular cells during late secretory and early proliferative phases. They observed a further increase in PTEN expression in decidual and glandular cells during early pregnancy. They proposed that PTEN might be one of the signaling proteins that estrogen and progesterone are acting to affect endometrial cell proliferation and/or apoptosis (14).

Chen et al investigated the possible involvement of the PTEN gene in the development of gestational trophoblasts and the pathogenesis of hydatidiform moles. They found that in partial and complete hydatidiform moles, the PTEN protein expression rate was significantly lower than in early placentas. However, partial hydatidiform moles, complete hydatidiform moles, and invasive moles were not significantly different in terms of PTEN protein expression. Their findings suggested that the regulation of PTEN expression may play an important role in the development of the early gestational trophoblast and in the pathogenesis of hyda-

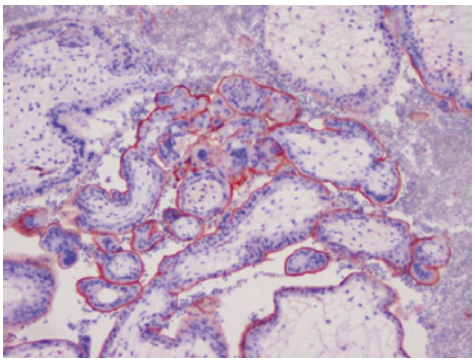

Figure 3. β1 integrin positivity around cell membranes in villous trophoblasts in spontaneous abortion (x100).

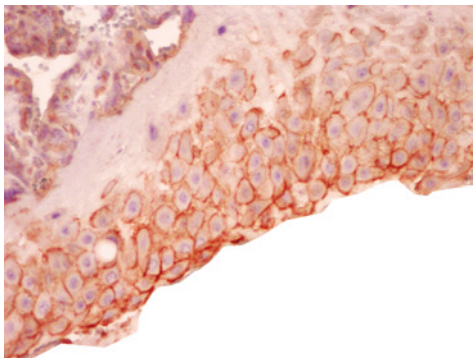

Figure 4. β1 integrin positivity around cell membranes in decidual cells in late pregnancy (x200).

tidiform mole, but not in its malignant transformation (22). PTEN protein may play an important role in trophoblast development. The early trophoblast cells possess the ability to proliferate and invade during embryo implantation, and form the chorion and placenta. These behaviours of the trophoblastic cells are regulated as the placenta maturates. PTEN protein may ensure normal development of gestational trophoblasts by controlling trophoblast proliferation and invasion (22). Chen et al postulated that down-regulated PTEN protein expression could lead to abnormal trophoblast proliferation, suggesting that lower PTEN expression is probably responsible for the pathogenesis of hydatidiform moles (22).

Ishioka et al analysed changes of apoptosis-related proteins induced by hypoxia in trophoblastic cells to clarify the mechanisms of hypoxia-induced apoptosis by using the PoweBlot, an antibody-based Western array. Hypoxia induced apoptosis was accompanied by increased expression of PTEN The bag-1 antisense oligonucleotide did not affect the expression of PTEN. Their findings were important to detect hypoxic stress of placenta, which leads to preeclampsia and other hypoxia-related obstetric complications (23).

Few articles examining expression of PTEN in placental tissues are available in the literature. To gain further insight on this subject, we have explored PTEN expression in placenta in light of spontaneous abortion. In our study, PTEN expression of villous trophoblasts was decreasing as the pregnancy advanced. PTEN expression decreased parallel to the development of placenta. Expression of PTEN in decidual cells was significantly stronger in placental tissues of spontaneous abortion than placental tissues from normal pregnancies at the first and third trimester. The up-regulation of PTEN expression in decidua may induce apoptosis and this may interfere with trophoblast proliferation and invasion. The abnormal PTEN expression may be a common pathway for pregnancy loss regardless of the etiology.

Integrins are adhesion-receptor proteins that mediates cell-cell and cell-extracellular interactions and plays a fundamental role in the regulation of gene expression, cell proliferation, and differentiation. These receptors link to the extracellular matrix proteins and transduce signals from the extracellular environment into the cell, activating cellular transduction pathways after binding with soluble mediators, cytokines, and growth factors (8).

During implantation and pregnancy, trophoblastic cells invade the decidua, simulating the process of stromal invasion by malignant cells. This is a complexly regulated process. It has been demonstrated that human endometrial and decidual cells express β1 integrin on their surfaces and this expression is a dynamic process throughout the menstrual cycle. β1 integrin expression in the human endometrium increases after implantation and remains high in the decidua during early pregnancy. It has been suggested that endometrial integrins play an important role in the process of implantation and decidualization (12,24). However, the role of β1 integrin variants in human decidua during early and late pregnancy remains to be clarified (8,10).

Lessey et al determined that the timing of expression of the α4β1 integrin framed the putative window of implantation and suggest a role in establishment of uterine receptivity (11).

Yoshimura et al investigated the expression of β1 integrin in human endometrium and decidua. They reported that the immunohistochemical distribution of β1 integrin demonstrated predominantly glandular epithelial staining in the proliferative phase, and stromal and glandular staining in the midsecretory phase (12).

Korhonen et al examined the distribution of the integrin subunits in human first and second trimester and term placentas. They stated that in first and second trimesters villi, β1 integrin subunit was detected in the stromal cells, whereas in the second and third trimesters it was expressed in villous trophoblast. Throughout placental development, decidual cells reacted prominently with anti-β1. They also demonstrated that the expression of integrin complexes is modulated during the differentiation of trophoblastic and decidual cells and suggested that integrin-mediated cell-basal membrane interactions may be important for placental development (15).

We have examined the expression of β1 integrin in villous trophoblasts and decidual cells of placental tissues from normal pregnancies at the first and third trimesters and spontaneous abortion. There was no significant difference between β1 integrin expression of three groups. Our results are not consistent with the findings of Korhonen et al (15). This may be because they used immunoflorescence microscopy and a panel of different antibody complexes. There was no significant correlation between β1 integrin expression and PTEN expression in villous trophoblasts and decidual cells.

Our findings suggest that altered patterns of PTEN expression may be associated with spontaneous abortion, but according to our results, it seems that β1 integrin does not contribute to this process as a signaling protein. Additional studies in larger series, including both PTEN antibody and specific β1 integrin antibody complexes will be needed to highlight this subject.

References

1. Li J, Yen C, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostat cancer. Science 28: 1943–1947.
2. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15: 356–362.
3. Li DM, Sun H (1997) TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor β. Cancer Res 57: 2124–2129.
4. Tamura M, Gu J, Tran H, Yamada KM (1999) PTEN gene and integrin signaling in cancer. J Natl Cancer Inst 91: 1820–1828.
5. Gimm O, Perren A, Weng L-P, Marsh DJ, Yeh JJ, Ziebold U, Gil F, Hinze R, Delbridge L, Lees JA, Mutter GL, Robinson BG, Komminoth P, Dralle H, Eng C (2000) Differential nuclear and cytoplasmic expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. Am J Pathol 156: 1693–1700.
6. Giri D, Ittmann M (1999) Inactivation of the PTEN tumor suppressor gene is associated with increased angiogenesis in clinically localized prostate carcinoma. Hum Pathol 30: 419–424.
7. Kumar V, Abbas AK, Fausto N, editors; (2004); Acute and chronic inflammation; In: Robbins Pathologic Basis of Disease; 7th ed; Elsevier Saunders China;. pp 47–86.
8. Vacca RA, Marra E, Loverro G, Maiorano E, Napoli A, Lovecchio M, Selvaggi L, Perlino E

(2003) Differential expression of β1c integrin messenger ribonucleic acid and protein levels in human endometrium and decidua during the menstrual cycle and pregnancy. J Clin Endocrinol Metab 88: 720–729.
9. Sueoka K, Shiokawa S, Miyazaki T, Kuji N, Tanaka M, Yoshimura Y (1997) Integrins and reproductive physiology: Expression and modulation in fertilization, embryogenesis, and implantation. Fertil Steril 67: 799–811.
10. Van der Linden PJQ, de Goeij AFPM, Dunselman GAJ, Erkens HWH, Evers JLH (1995) Expression of cadherins and integrins in human endometrium throughout the menstrual cycle. Fertil Steril 63: 1210–1206.
11. Lessey BA, Castelbaum AJ, Buck CA, Lei Y, Yowell CW, Sun J (1994) Further characterization of endometrial integrins during the menstrual cycle and pregnancy. Fertil Steril 62: 497–506.
12. Yoshimura Y, Miyakoshi K, Hamatani T, Iwahashi K, Takahashi J, Kobayashi N, Sueoka K, Miyazaki T, Kuji N, Tanaka M (1998) Role of beta 1 integrins in human endometrium and decidua during implantation. Horm Res 50 Suppl 2: 46–55.
13. Mutter GL, Lin M-C, Fitzgerald JT, Kum JB, Eng C (2000) Changes in endometrial PTEN expression throughout the human menstrual cycle. J Clin Endocrinol Metab 85: 2334–2338.
14. Kayışlı ÖG, Kayışlı ÜA, Al-Rejjal R, Zheng W, Lüleci G, Arıcı A (2003) Regulation of PTEN (phosphatase and tensin homolog deleted on chromosome 10) expression by estradiol and progesterone in human endometrium. J Clin Endocrinol Metab 88: 5017–5026.
15. Korhonen M, Ylänne J, Laitinen L, Cooper HM, Quaranta V, Virtanen I (1991) Distribution of the α1-α6 integrin subunits in human developing and term placenta. Lab Invest 65: 347–356.
16. Torres J, Navarro S, Roglá I, Ripoll F, Lluch A, García-Conde J, Llombart-Bosch A, Cervera J, Pulido R (2001) Heterogenous lack of the tumour suppressor PTEN protein in human neoplastic tissues. Eur J Cancer 37: 114–121.
17. Taniyama K, Goodison S, Ito R, Bookstein R, Miyoshi N, Tahara E, Tarin D, Urquidi V (2001) PTEN expression is maintained in sporadic colorectal tumors. J Pathol 194: 341–348.
18. Manzotti M, Dell'Orto P, Maisonneuve P, Fornaro M, Languino LR, Viale G (2000) Down-regulation of beta(1C) integrin in breast carcinomas correlates with high proliferative fraction, high histological grade, and larger size. Am J Pathol 156: 169–174.
19. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP (1998) Pten is essential for embryonic development and tumour suppression. Nat Genet 19: 348–355.
20. Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, Ho A, Wakeham A, Itie A, Khoo W, Fukumoto M, Mak TW (1998) High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. Curr Biol 8: 1169–1178.
21. Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, Cordon-Cardo C, Catoretti G, Fisher PE (1999) Mutation of *Pten/Mmac1* in mice causes neoplasia in multiple organ systems. Proc Natl Acad Sci 96: 1563–8.
22. Chen H, Ye D, Xie X, Lu W, Zhu C, Chen X (2005) *PTEN* promoter methylation and protein expression in normal early placentas and hydatiform moles. J Soc Gynecol Investig 12: 214–217.
23. Ishioka S, Ezaka Y, Umemura K, Hayashi T, Endo T, Saito T (2006) Proteomic analysis of mechanisms of hypoxia-induced apoptosis in trophoblastic cells. Int J Med Sci 4: 36–44.
24. Shiokawa S, Yoshimura Y, Nagamatsu S, Sawa H, Hanashi H, Oda T, Katsumata Y, Koyama N, Nakamura Y (1996) Expression of beta 1 integrins in human endometrial stromal and decidual cells. J Clin Endocrinol Metab 81: 1533–1540.

Corresponding author:
Cigdem Tokyol
Afyon Kocatepe Üniversitesi
Ali Çetinkaya Kampüsü
Uygulama ve Araştırma Hastanesi
Patoloji Bölümü
Afyonkarahisar, TURKEY
Ph: 90 272 2142065
Fax: 90 272 2133066
E-mail: ctokyol@yahoo.com