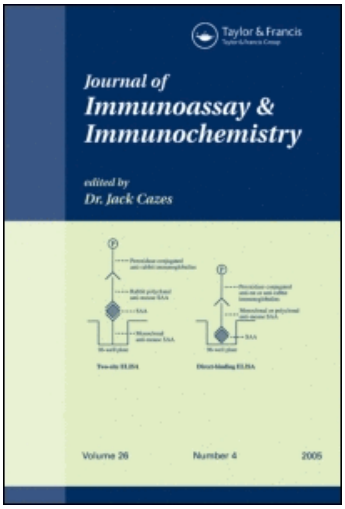

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EDA Fibronectin Isoform of Amniotic Fluid in Relation to Normal Pregnancy Stages and to Pregnancies Complicated by Fetal Postmaturity Syndrome

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Abstract: The relative expression of the EDA region in fibronectin (FN) was determined by ELISA, using specific monoclonal antibody anti-EDA-FN, in the amniotic fluid samples derived from: 2nd trimester, early 3rd trimester, term, and post-term pregnancy, delivery at 37–40 weeks and at 41–42 weeks, as well as pregnancies complicated by fetal postmaturity. The expression of EDA-FN isoform was almost on the same level from the 2nd trimester to the 3rd trimester including term and post-term pregnancy. However, its relative amount significantly decreased in delivery groups and was significantly higher in the pregnancies with fetal postmaturity syndrome.

Keywords: Amniotic fluid, EDA-fibronectin isoform, Fibronectin, Postmaturity, Pregnancy

INTRODUCTION

Fibronectin (FN) is macromolecular, multifunctional, and multidomain adhesive glycoprotein, abundant in extracellular matrices and in body

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fluids. FN consists of three types of repeating modules (I, II, and III) arranged into unique cellular, collagen, fibrin, and heparin domains that specifically interact with extracellular matrix components. It is known that FN has multiple isoforms produced by alternative splicing of the primary gene transcript at three distinct regions, termed EDA, EDB, and variable IIICS domains. They are generated by the insertion of extra III segments in the cellular domain of FN.[1,2] Plasma FN, produced by hepatocytes, lacks A and B domains, but it can possesses the IIICS domain. Cellular FN is produced by a number of cell types and may contain various combinations of spliced segments EDA, EDB and IIICS.[3]

FN containing EDA (EDA-FN) is mainly overexpressed in tissues after stimulation by growth factors, cytokines, hormones, and stress factors. It is highly expressed during embryogenesis, but only slightly expressed in adult tissue, except in some pathological conditions such as wound healing, liver fibrosis, and in many proliferative processes.[4] The biological function of EDA-FN is still poorly understood. An alternatively spliced EDA segment renders FN a better substrate for cell interaction than has been found in the cellular FN domain. EDA-FN can modulate the structural and functional properties of the local extracellular matrix: it influences migration, proliferation, and differentiation of cells;[3,5] it can also regulate fibronectin-dependent cell cycle progression and mitogenic signal transduction.[6] The cellular adhesion is known to occur, respectively, via cellular FN domain and CS-1 segment of FN through integrins $\alpha 5\beta 1$ and $\alpha 4\beta 1$,[7] whereas the adhesion via EDA segment is regulated by a new mechanism, through integrins $\alpha 9\beta 1$ and $\alpha 4\beta 1$.[8]

FN has been found to be associated with the pericellular and extracellular matrix of cultured amniotic fluid cells, which explains its presence in amniotic fluid.[9] It is thought that amniotic fluid FN is locally produced rather than derived from plasma and, in contrast to plasma, contains EDA segment and variable oncofetal epitope.[10] The detection of oncofetal FN isoform in the second and early third trimester has been found to be associated with a risk of preterm delivery,[11] but not with prolonged pregnancy.[12]

In the present work, we have presented a dynamic expression of EDA domain in amniotic fluid FN (EDA-FN) in relation to the progression of normal pregnancy, as well as in pregnancies complicated by fetal postmature syndrome. The relative level of EDA-FN was estimated by ELISA using anti-EDA-FN specific monoclonal antibody in the following groups of amniotic fluid samples: 2nd and the early 3rd trimesters, term pregnancy, delivery at 37–40 weeks and at 41–42 weeks, post-term pregnancies, as well as pregnancies complicated by fetal postmaturity.

EXPERIMENTAL

Patients and Sampling

Randomly chosen samples of amniotic fluid were taken from 203 pregnant women (21–44 years old) with gestational ages between 14 and 42 weeks who had been receiving prenatal care at the Department and Clinic of Reproduction and Obstetrics of Wrocław Medical University (Poland) in the years 2000–2001.

Accurate gestational dating was defined as a knowledge of the most recent menstrual period with confirmation by ultrasonographic evaluation performed during the first and second trimesters of pregnancy. All samples were collected with the informed consent of the individual women and the study was approved by a local ethics committee. The samples used were the remaining fluid after the routine diagnostic procedures were performed. Amniotic fluid was obtained by transabdominal amniocentesis under ultrasonographic guidance or by transvaginal amniotomy during delivery at term. Vaginal and cervical secretions were ruled out before the puncture. Immediately after amniocentesis all amniotic fluid samples were centrifuged at 3,000 g for 20 min., aliquoted, and stored at $-80^{\circ}C$ until used. Frozen samples were thawed at $20^{\circ}C$ before being used. Amniotic fluid samples contaminated with blood or meconium were discarded.

From the total 203 collected samples, 122 amniotic fluid samples were selected, based on the patients' diagnoses. In 115 cases, delivered infants were healthy, and 7 infants appeared postmature at birth. The samples of amniotic fluid were divided into the following groups:

1. "2nd trimester", n = 19: from 13 to 20 weeks of gestation. The samples were drawn in order to establish fetal karyotyping. These women delivered healthy newborns at term without malformations or chromosomal abnormalities;
2. Early "3rd trimester", n = 14: from 32–37 weeks of pregnancy;
3. Term pregnancy, n = 26: from 38–40 weeks of pregnancy;
4. "Post-term pregnancy", n = 19: from 41–42 weeks of pregnancy. This group comprises 12 pregnancies ended by Cesarean section and 7 pregnancies whose delivered fetus by spontaneous vaginal delivery lasted from 61 h up to 200 h.
5. "Delivery", n = 23: from 37–40 weeks of pregnancy: Healthy fetuses, spontaneous vaginal delivery at up to 3 hours.
6. "Delivery", n = 14: from 41–42 weeks of pregnancy. Healthy fetuses, spontaneous vaginal delivery up to 3 hours.
7. "Postmature pregnancy", n = 7: from 40–42 weeks. Of these pregnancies, 5 ended by Cesarean section and two women delivered children by spontaneous vaginal delivery up to 3 hours. All infants showed

postmaturity syndrome at birth: they appeared wasted, dry skinned, and showed signs of subcutaneous tissue loss.

All samples of 1–6 groups were from normal gestation; pregnancies ended without any complications and healthy fetuses were delivered without symptoms of infection.

Additionally, blood plasma samples taken from 10 healthy non-pregnant volunteers (22–35 years old), as well as from 49 pregnant women of 15–42 weeks of gestation were included in the test as a negative control.

Expression of EDA Region in FN

The expression of the EDA region in FN (EDA-FN) was determined in relation to a constant FN concentration in amniotic fluid. This was done to avoid the false data of EDA-FN determination, which could result from the alterations of amniotic fluid volume during pregnancy. The relative amount of EDA-FN was determined by sandwich-type ELISA and the experimental details are as follows: the monoclonal antibody anti-cellular EDA-FN (Chemicon International, MAB 1940; 100 μL of diluted 1:10,000) was immobilized on wells of a microtiter plate (high avidity plates, Nalge Nunc International, USA) for 2 h at 37°C. After washing and blocking (1% bovine serum albumin, Sigma; St. Louis, MO, USA) procedures, the wells of microtiter plate were incubated with 100 μL of amniotic fluid, respectively adjusted by a proper dilution with TBS-0.1% Tween-20 to a final FN concentration of 500 μg/mL, measured previously by ELISA using monoclonal antibody (FN 30-8; M010, TaKaRa Biomedicals Inc., Tokyo, Japan) directed to the cellular domain of FN as described earlier.[13] Next, after washing, the wells were incubated for 1 hour at 37°C with 100 μL of rabbit polyclonal anti-FN antibodies (Sigma, St. Louis, MO, USA, diluted 1:10,000). Following a washing step, the plate was incubated with 100 μL of secondary antibodies, peroxidase-conjugated goat anti-rabbit immunoglobulins (Sigma; St. Louis, MO, USA, diluted 1:10,000). The amount of FN was assayed by colorimetry using o-phenylenediamine dihydrochloride/H_2O_2 as the enzyme substrate and the absorbance was measured in a Stat Fax 2100 Reader at 492 nm and at 630 nm as the reference filter. The result was given in absorbance units (AU). All ELISA immunobinding and washing steps were carried out in TBS containing 0.1% Tween 20, pH 7.3.

Statistics

For statistical analysis the Statistica 6.0 computer program (StatSoft Inc., Tulsa, OK, USA) was used. Data were expressed as mean ± standard

deviation (SD). Statistical significance between groups was calculated by the Mann-Whitney test. A p-value of less than 0.05 was required to reject the null hypothesis.

RESULTS AND DISCUSSION

Linnala et al.[10] have reported that amniotic fluid derived from 15–16 weeks of gestation, but not blood plasma of pregnant women, contains the EDA-FN isoform of fibronectin (^{EDA}FN). Results of our work are in accordance with this and, moreover, EDA-FN was found in all samples of amniotic fluid throughout 14–42 weeks of gestation (Table 1). In contrast, only traces or undetectable amounts of EDA-FN were observed in blood plasma of pregnant (0.06 ± 0.07 AU) and non-pregnant women (0.07 ± 0.02 AU). The relative amount of EDA-FN increased negligibly

Table 1. Expression of EDA-FN in amniotic fluid.

No of groups	Groups of amniotic fluid and age of pregnancy	Number of samples	Amniotic EDA-FN (*AU)
1.	2nd trimester 13–20 weeks	19	0.39 ± 0.1
2.	3rd trimester 32–37 weeks	14	0.45 ± 0.2
3.	Term pregnancy 38–40 weeks	26	0.47 ± 0.2^{1} $p < 0.01$
4.	Postterm pregnancy 41–42 weeks	19	0.47 ± 0.2^{1} $p < 0.02$
5.	Delivery 37–40 weeks	23	0.31 ± 0.1
6.	Delivery 41–42 weeks	14	0.32 ± 0.2
7.	Postmature pregnancy 40–42 weeks	7	$0.82 \pm 0.25^{1,2,3}$ $^{1}p < 0.0005$ $^{2}p < 0.005$ $^{3}p < 0.01$
8.	Blood plasma of		
	– pregnant 15–42 weeks	49	0.06 ± 0.07
	– non-pregnant women	10	0.07 ± 0.02

*The relative amount of EDA-FN was given in value of Δ absorbance units (AU) obtained in FN-ELISA for 500 ng of FN. Details see in Materials and Methods. Significantly different from: [1]delivery groups of 37–40 weeks and 41–42 weeks; [2]term pregnancy and 3rd trimester; [3]postterm pregnancy.

with the advance of pregnancy from the 2nd trimester (0.39 ± 0.1 AU), throughout the 3rd trimester (0.45 ± 0.25 AU), to the term pregnancy (0.47 ± 0.2 AU). Also, in the post-term pregnancy at 41–42 weeks, the EDA-FN expression was found to be on the same level (0.47 ± 0.2 AU). However, during the delivery at 37–40 weeks, as well as at 41–42 weeks, the relative amount of EDA-FN decreased significantly ($p < 0.01$) to the values of 0.31 ± 0.1 AU and 0.32 ± 0.2 AU, respectively. Described above alterations in amniotic EDA-FN expression during pregnancy were probably related to the biosynthesized and secreted by the placenta steroid hormones and cytokines. Such biomolecules are important components of communication network, operating within the feto-maternal interface to ensure the successful establishment of pregnancy, and to initiate a delivery.[14] Moreover, they are known to participate in a cascade of cellular events that could significantly stimulate gene expression, leading to an alternative splicing of single gene transcript[4] and, thus, to the expression of EDA-FN isoform.

In contrast, the expression of the amniotic EDA-FN increased to the value of 0.82 ± 0.2 AU in samples of amniotic fluid derived from pregnancy complicated by fetal postmature syndrome, when compared with delivery ($p < 0.0005$) and perinatal ($p < 0.005$) groups, respectively (Table 1). A clinical postmaturity syndrome of a fetus is thought to be a consequence of failing placental function[15] and FN containing alternatively spliced ED-A domain is one of active participants of fibrous network of placental extracellular matrices,[16] thus EDA-FN might be released from matrix to amniotic fluid during placental dysfunction.

CONCLUSION

1. Fibronectin containing an alternatively spliced EDA region is a normal constituent of amniotic fluid throughout 14–42 weeks of gestation.
2. An increased expression of amniotic EDA-FN, observed in pregnancies complicated by fetus postmaturity reflects, probably, cellular remodeling processes occurring between extracellular matrices of placenta, and chorionamniotic and/or chorionic-decidual membranes.
3. Our results, however preliminary, suggest that the determination of EDA-FN isoform by simple ELISA test could be of importance in pregnancy care monitoring, particularly in a management of post-term pregnancy. A decrease could announce a spontaneous delivery and an increase could predict a risk to a fetus of the postmaturity syndrome.

ABBREVIATIONS

FN, fibronectin; EDA-FN, fibronectin with expressed EDA domain.

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