CEOG Clinical and Experimental Obstetrics & Gynecology

Disturbed angiogenesis in intrauterine growth restriction-compromised placentas at term

P. Bolehovska[1], D. Driak[1], M. Halaska[1], B. Asfaw[2], J. Novotny[3], I. Svandova[3]

[1]Department of Gynaecology and Obstetrics, First Faculty of Medicine and Hospital Bulovka, Charles University, Prague
[2]Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague
[3]Department of Physiology, Faculty of Science, Charles University, Prague (Czech Republic)

Summary

Purpose of investigation: The aim of this study was to compare levels of selected angiogenesis-related proteins in intrauterine growth restriction (IUGR)-compromised and normal placentas at term. *Materials and Methods:* Stromatal tissue of placental villi of gestationally matched IUGR patients and controls (both groups of n=32, gestational age between 39+0 and 40+6 gtt) was separated by microdissection. After separation, placental tissue was homogenised on ice (2´10 min, 2,000 rpm) and centrifuged (2´5 min, 5,000 rpm). Proteins of supernatant were fractionated by 8% or 10% SDS-PAGE and Tie-1, Tie-2, VEGFR-1, VEGFR-2, and MMP-2 levels were measured by Western blot using appropriate primary and secondary antibodies. *Results:* In the IUGR group, compared to the control group, there was a significantly lower level of Tie-1 (67.1% of controls, $p < 0.001$), VEGFR-1 (72.4% of controls, $p < 0.001$), VEGFR-2 (68.5% of controls, $p = 0.001$), and a significantly higher level of Tie-2 (119% of controls, $p = 0.023$). MMP-2 was increased in the IUGR group (127% of controls, $p < 0.001$). *Conclusions:* Placental levels of Tie-1, Tie-2, VEGFR-1, and VEGFR-2 are altered in pregnancies complicated by IUGR. Compared with that in normal pregnancies, the level of MMP-2 was upregulated. The present authors speculate that the results of this study represent the angiogenic imbalance observed in IUGR pregnancies; thereby resulting in poor and disturbed angiogenesis underlying delayed development of the fetus.

Key words: IUGR; Placenta; Angiogenesis; Angiogenic factors.

Introduction

The placenta is a unique organ that develops and functions only during pregnancy. The normal development of the placenta is mastered by angiogenic growth factors. Successful placentation depends on the formation of an efficient vascular network established by processes of vasculogenesis and angiogenesis [1, 2]. Impaired placentation is associated with many pregnancy pathologies, including intrauterine growth restriction (IUGR) [3].

IUGR is a severe pregnancy complication, resulting in birth of a fetus with estimated weight below the 10th percentile for its gestational age, and with abdominal circumference below the 2.5th percentile [4]. The IUGR incidence range is estimated to be 3-10%, with respect to the examined newborn population, geographic location, and standard reference growth curves [5]. The imbalance between pro-angiogenic and anti-angiogenic factors is suggested to be a key factor in the development of IUGR [6-8].

The most well-characterised of four ligands of the angiopoietin system are angiopoietin 1 (Ang-1) and angiopoietin 2 (Ang-2). These are crucial angiogenic growth factors acting in the later stages of placental angiogenesis [9, 10]. Their corresponding receptors include tyrosine kinase receptor Tie-2, which is activated by Ang-1 and blocked by Ang-2 [11, 12], and Tie-1 receptor, often considered an orphan receptor [13]. During pregnancy, angiopoietins are expressed in placenta even from the first trimester [9]. Ang-1 promotes endothelial cell survival, endothelial cell migration, and the remodelling of vessels [14, 15], whereas Ang-2, as the natural antagonist of Ang-1, regulates endothelial cell apoptosis and vessel regression, thus facilitating vascular endothelial growth factor (VEGF)-induced neovascularization [16]. In general, angiopoietins seem to act as co-players to the VEGF system and have a major role in the later stages of angiogenesis [17].

VEGF was one of the first angiogenic factors identified, and is generally recognised as an important regulator of both normal and pathological angiogenesis [18, 19]. In pregnancy, VEGF is expressed by human villous and extravillous trophoblasts, securing there the formation of new blood vessels by affecting proliferation, migration, and metabolic activity of trophoblasts [7, 8, 20]. Upon receiving an angiogenic stimulus, endothelial cells follow the stimulatory gradient and migrate into a previously avascular region. The endothelial sprouting involves remodelling of the extracellular matrix (ECM) by the activity of metalloproteinases (MMPs) [21, 22]. MMPs comprise a family of over 20 human structurally related zinc-dependent endopeptidases, which have broad substrate specificity. Among MMPs, gelatinases (MMP-2 and -9) are capable of

Revised manuscript accepted for publication May 31, 2017

degrading denatured collagens and basement membrane components and remodelling ECM [23, 24]. Several studies have shown that alterations in MMP expression and activity may be associated with IUGR [25-27].

The studies regarding the role of angiogenic factors in IUGR-compromised pregnancies have usually examined the circulating levels of angiogenic factors and their respective soluble receptors in maternal serum. Previous studies that have investigated the placental expression of angiopoietin and VEGF-family receptors in IUGR pregnancies are not numerous, and their results are inconsistent. Moreover, the studies have usually used rather small sample sizes and have often not defined whether IUGR was related to preeclampsia (PE) or could be classified as idiopathic. A study by Geva *et al.* demonstrated that the placental Tie-2 mRNA expression was not changed in pregnancies compromised by PE with IUGR in the third trimester [28]. Findings of Kappou *et al.* showed a significant increase in the placental expression of Tie-2 in PE-IUGR pregnancies [29]. Semczuk *et al.* observed higher mRNA expression for VEGFR-1 and VEGFR-2 genes in 25 IUGR placentas [30]. Vuorela and Halmesmäki performed immunohistochemical analysis of Tie-1, Tie-2, VEGFR-1, and VEGFR-2 in five IUGR-compromised placentas that exhibited the highest VEGFR-2 immunoreactivity in placentas with IUGR [31]. Rajakumar *et al.* investigated mRNA levels of membrane and soluble VEGF receptor-1 proteins in normotensive pregnancies complicated by late-onset IUGR, with results indicating no difference between normal and IUGR placentas [32]. On the other hand, Helske *et al.* reported increased placental expression of VEGFR-1 in placentas with fetal growth restriction [33].

The purpose of this study was to examine levels of placental VEGFR-1, VEGFR-2, Tie-1, Tie-2, and MMP-2 proteins in IUGR-compromised and normal placentas at term.

Materials and Methods

Stromatal tissue of placental villi was obtained from gestationally-matched IUGR patients and controls. The study population was comprised of placentas from 32 uncomplicated and 32 IUGR pregnancies of gestational age between 39+0 and 40+6 weeks. Placentas from twin pregnancies, infants with congenital anomalies, and those from pregnancies with maternal complications such as chronic hypertension or diabetes mellitus were excluded from the study. The gestational age in days was defined by the last menstrual period. The group of IUGR-compromised pregnancies was defined by the birth weight of newborns below the 10^{th} percentile and by an elevated pulsatility index (PI) in the umbilical arteries (> 2 SD). Clinical characteristics of the groups are shown in Table 1. Written consent was obtained from all subjects of the study.

Placental samples were collected at delivery from both groups. The tissue specimens were taken immediately after the extraction of the placenta from the uterus. Several basal plate biopsy specimens of the maternal-foetal interface (1.5×1.5×1 cm in diameter) were randomly excised from each placenta in a way that each sample contained the decidua basalis and villous placenta. Areas involving calcification or infarcts were avoided. The tissue specimens were immediately frozen at -80°C and stored until processing.

The obtained tissue was brought to 0°C and washed in cold normal saline to eliminate any contaminating blood. Endometrial stroma was prepared by microdissection under optic control. To isolate cytosol fractions, the isolated stroma was homogenized in ten volumes of the homogenization buffer (20.0 mM Tris-HCl; 2.5 mM EDTA; 50.0 mM NaF; 10.0 mM $Na_4P_2O_7$, 1% Triton X-100; pH 7.4) containing a complete protease inhibitor cocktail for mammalian tissues (AEBSF - aprotinin, leupeptin, bestatin, pepstatin A, and E-64) on ice by using a tight teflon-glass homogenizer (3´10 min at 2,000 rpm). The homogenate was centrifuged at low-speed at 10,000 g for 2´10 min at 4°C. The resultant supernatant and pellet were subsequently separated. Supernatant was then collected and snap frozen in liquid nitrogen and stored at –80°C until use. The concentration of the proteins was determined by the method of Lowry.

The sample containing 25 μg of protein was solubilized in Laemmli buffer (50 mM Tris/HCl, pH 8.0, 6% (w/v) dithiothreitol, 5% (w/v) SDS, 0.005% (w/v) Bromphenol Blue). The proteins were resolved by standard SDS-PAGE (8% gels for Tie-1, Tie-2, VEGFR-1 and VEGFR-2, and 10% gels for MMP-2). The electrophoresis was run at 200 V for one hour, using a mini protean II gel kit. Proteins were then transferred to a nitrocellulose membrane using a wet apparatus. The membrane was blocked for one hour in 5% fat-free milk in PBST buffer (PBS containing 0.05% (v/v) Tween 20) at room temperature. Subsequently, the membrane was incubated for two hours at room temperature with appropriate primary antibody in 1% fat-free milk in PBS-T buffer at room temperature. The following primary antibodies were used: against Tie-1 (sc-365257, 1:1000), against Tie-2 (sc-9026, 1:1000), against VEGFR-1 (D-2, sc-271789, 1:1000), VEGFR-2 (D-8, sc-393163, 1:1000), and against MMP-2 (sc-10736, 1:500). After the primary antibody was removed, the blot was washed for 3´10 min in PBS-T buffer. Subsequently, the membrane was incubated with appropriate secondary antibody (1:15 000-20 000) in 1% fat-free milk PBS-T buffer for one hour at room temperature. After removing the secondary antibody, the membrane was extensively washed three times for ten minutes in PBS-T buffer and the blot was visualized by ECL.

The expression levels of proteins were analysed and quantified by scanning densitometry. Blots were scanned for quantification of band intensity. The expression level corresponded to the number of black pixels scanned of each band. The results were expressed as optical density (OD) in arbitrary units. Statistical analysis was performed using the SigmaStat 3.5 program. The unpaired *t*-test was used. For categorical variables, a Chi-square test was performed. When indicated, a non-parametric Mann-Whitney Rank Sum Test for continuous variables was used for the group comparisons. *P*-values less than 0.05 were considered statistically significant.

Results

The present study sample included 32 normotensive pregnancies complicated by IUGR and 32 normotensive controls. The characteristics of the study groups are shown in Table 1. Birth weight at delivery and placental mass differed ($p < 0.001$) between the analysed groups, while no statistical differences were found regarding maternal age, gestational age at delivery, and child gender.

Table 1. — *Characteristics of the study sample.*

	IUGR	Control	*p*-value
Cases (n)	32	32	
Maternal age (years)	30.3±4.3	30.9±4.5	0.571
Gestational age at delivery (days)	273.4±7.1	275.1±7.9	0.423
Birth weight (g)	2415.0±276.6	3138.8±105.4	<0.001
Child gender (male, %)	50.9±0.5	52.7±0.5	0.852[a]
Placental weight (grams)	375.0±102.0	546.9±44.5	<0.001

Data are presented as mean ± SD or percentage. [a] Chi-square test for categorical variables. A non-parametric Mann-Whitney Rank Sum Test for continuous variables was used for the group comparisons.

Table 2. — *Comparison of Tie-1, Tie-2, VEGFR-1, VEGFR-2, and MMP-2 expression levels in control and IUGR placentas.*

	IUGR (% of Controls)	*p*-value
Tie-1	67.1	<0.001
Tie-2	119	0.023
VEGFR-1	72.4	<0.001
VEGFR-2	68.5	0.001
MMP-2	127	<0.001

Comparison of protein levels quantified by Western blots revealed differences in all studied protein between the IUGR and control groups. The results are summarised in Table 2. The authors found that expression levels of Tie-1 (Figure 1a, Table 2) and both VEGF receptors (Figure 1c and 1d, Table 2) were significantly decreased in the IUGR group. Comparison revealed increased expression of Tie-2 in samples from IUGR placentas compared to the control group (Figure 1b, Table 2). The same trend was found for MMP-2, with 127% of expression level of the control group (Figure 1e, Table 2).

Discussion

The broadly accepted pathophysiological concept for IUGR-compromised pregnancies implies an imbalance of angiogenic factors and their impaired cooperation. The present findings demonstrate that in pregnancies complicated with IUGR, placental VEGFR-1, VEGFR-2, and Tie-1 levels were decreased, whereas there was an increase in the levels of Tie-2 and MMP-2.

Previous studies have shown that angiopoetic factors play a key role in a number of endothelial and non-endothelial events during placentation. However, literary data still give an inconsistent and sometimes contradictory picture of placental levels of angiopoetic factors in pregnancies with impaired placentation. The studies regarding the role of angiogenic factors in placentas of IUGR-compromised pregnancies have usually examined their respective mRNA expression [28-30, 32, 34], and just a few studies have investigated the placental levels of angiopoetic factors in IUGR pregnancies by Western blot [33] or immunohistochemistry [31, 33].

The present authors have demonstrated that levels of placental VEGFR-1, VEGFR-2, and Tie-1 tend to be lower in normotensive IUGR pregnancies at term. These findings differ somewhat with those of several studies. In a Western blot study by Helske *et al.*, VEGFR-1 but not the other receptors showed increased expression in placental syncytiotrophoblasts from 50% of patients with compromised pregnancies. Levels of other receptors remained unchanged [33]. In their immunohistochemistry study, Vuorela and Halmesmäki described decreased VEGFR-1 immunoreactivity in the myometrial vascular smooth muscle cells of women with IUGR. However, they observed the strongest VEGFR-2 immunoreactivity in the IUGR myometrial cells [31]. On the other hand, Zhou *et al.* showed by immunohistochemistry and Western blotting, decreased cytotrophoblast VEGF-A and VEGFR-1 levels in PE pregnancies. Decreased levels of VEGFR-1 were counterbalanced with increased levels of soluble receptor form sFlt-1, which is a hallmark of compromised pregnancies [35]. As it is well known that sFlt-1 directly attenuates VEGFR-2 expression [36], decreased levels of VEGFR-1 in the present study can also be linked with an expected sFlt-1 increase, and thus with a VEGFR-2 decrease in turn. The supposed phenomenon of that inverse action acts through heterodimerization of sFlt-1 with VEGFR-2 and subsequent inhibition of VEGFR-2 expression [36].

Studies dealing with placental mRNA expression usually depict different levels of angiogenic factors and their respective receptors in comparison with IUGR-compromised pregnancies. Semczuk *et al.* observed higher mRNA expression for VEGFR-1 and VEGFR-2 genes in 25 IUGR placentas [30]. On the other hand, Andraweera *et al.* concluded that the level of placental mRNA of the VEGF family of angiogenic growth factors is reduced in small for gestational age infants [34]. Moreover, Rajakumar *et al.* investigated mRNA levels of membrane and soluble VEGF receptor-1 proteins in normotensive pregnancies complicated by late-onset intrauterine growth restriction, with results indicating no difference between normal and IUGR placentas [32]. However, results obtained by Western blotting and mRNA expression should be compared with maximal caution. A gene's mRNA level does not necessarily predict its protein level, and correlation of expression levels can vary from poor to moderate [37]. Nevertheless, the present authors suggest that their results' discrepancy with broadly comparable Western blot or immunohistochemistry studies was likely due to possible heterogeneity of examined placental tissue among studies.

The present results showed an increase in the levels of Tie-2 and MMP-2. Once again, regarding Tie-2 levels, sim-

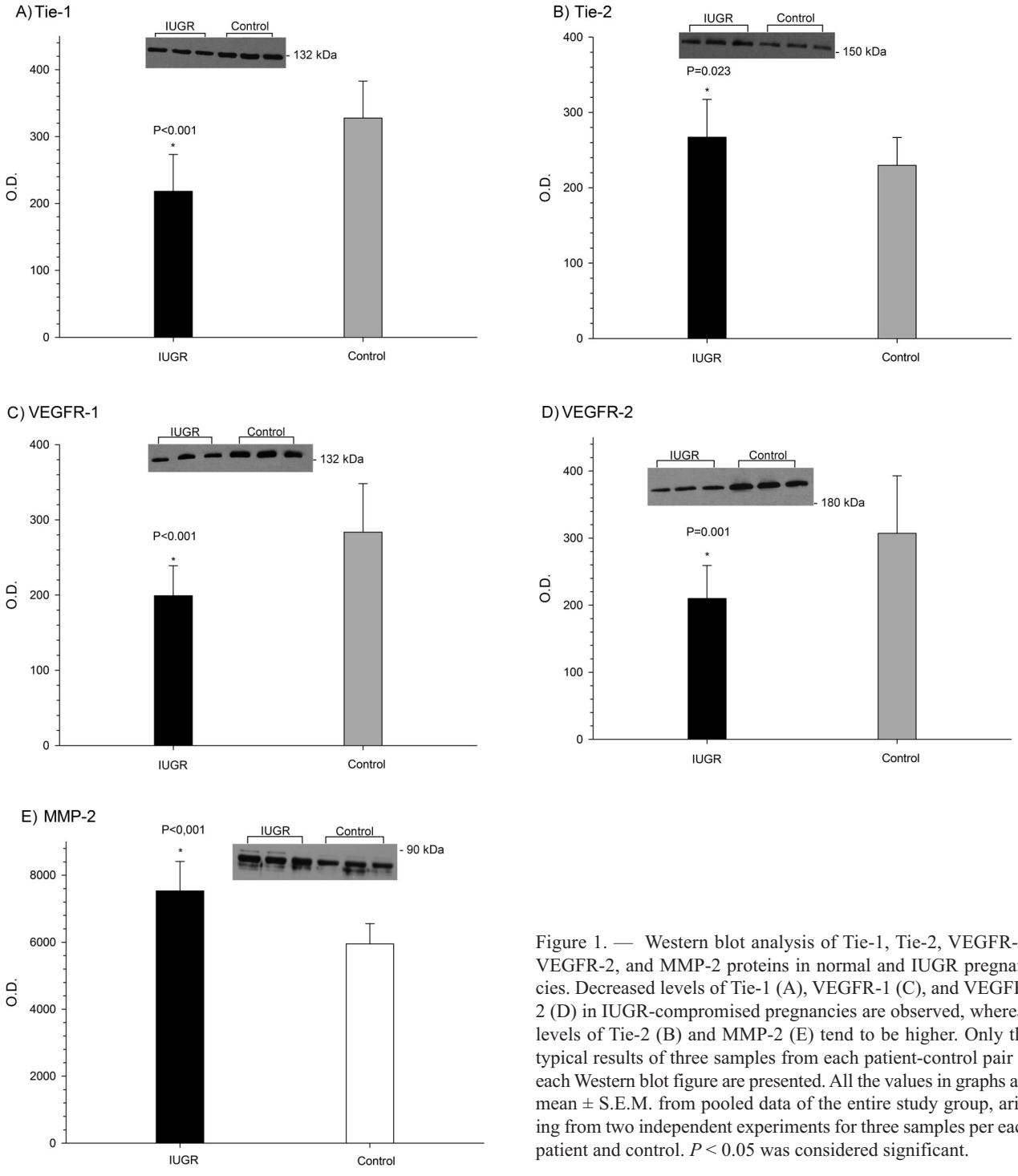

Figure 1. — Western blot analysis of Tie-1, Tie-2, VEGFR-1, VEGFR-2, and MMP-2 proteins in normal and IUGR pregnancies. Decreased levels of Tie-1 (A), VEGFR-1 (C), and VEGFR-2 (D) in IUGR-compromised pregnancies are observed, whereas levels of Tie-2 (B) and MMP-2 (E) tend to be higher. Only the typical results of three samples from each patient-control pair in each Western blot figure are presented. All the values in graphs are mean ± S.E.M. from pooled data of the entire study group, arising from two independent experiments for three samples per each patient and control. $P < 0.05$ was considered significant.

ilar heterogeneity exists among the few published studies. Vuorela and Halmesmäki reported stable Tie-2 immunoreactivity between IUGR and control groups [31]. A study by Geva *et al.* also demonstrated that placental Tie-2 mRNA expression was not changed in pregnancies compromised by PE with IUGR in the third trimester [28]. On the other hand, Kappou *et al.* showed a significant increase in the placental expression of Tie-2 in PE and IUGR pregnancies. The changes were more significant in pregnancies further complicated with IUGR than in PE [29]. In a longitudinal study by Bolin *et al.*, the authors demonstrated upregulation of Ang-2, a naturally occurring disruptor of embryonic vascular development mediated by Tie-2, in maternal serum of PE pregnancies during the whole gestation [38]. Those

particular observations are in accord with the present findings; however, the direct comparison of this data with previously published findings is difficult, due to the diversity of methods and tissue used. Nevertheless, Ang-2 is supposed to act as the dynamic up-regulator of the Ang-Tie2 axis in numerous diseases including inflammation [39, 40]. In a recent study, induced vascular inflammation in pregnant mice resulted in IUGR and even intrauterine fetal death [41]. One can speculate that increased Ang-2 levels, mirroring increased Tie-2 levels, reflect unrecognised vascular inflammation in the present IUGR placenta samples.

The number of studies depicting alterations in placental MMP expression and activity associated with IUGR is even more limited [25-27, 42]. By using methods of Western blotting and immunohistochemistry, Zhu *et al.* studied levels of MMPs in the pathogenesis of PE and fetal growth restriction. They showed that the expression of MMP-2 was downregulated in villous tissues of PE and IUGR cases [42]. Merchant *et al.* examined MMP-2 activity in explants from IUGR pregnancies compared with normal pregnancies, by zymography, and found reduced MMP-2 activity in explants cultured in 20% oxygen [27]. In contrast, the zymographic and ELISA results of Mckirdy and Marks showed no significant difference in MMP-2, MMP-9 or TIMP-2 levels in placentas of IUGR pregnancies [25]. No significant differences were also found between activity of total MMP-2 and total MMP-9 in first trimester placental bed biopsies of IUGR-complicated and uncomplicated pregnancies [26]. The present findings described the increase in MMP-2 level. MMPs are recognised for their key role in inflammation, repair, and remodelling processes [43]. As MMPs are induced in inflammation, in connection with higher Tie-2 levels discussed above, and as one possible explanation, the present authors postulate that increased MMP-2 levels in this IUGR group are related to unrecognised vascular inflammation.

Conclusion

In summary, the present authors have demonstrated lower levels of VEGFR-1, VEGFR-2, and Tie-1 proteins and increased levels of Tie-2 and MMP-2 in IUGR-compromised placentas. This study provides additional Western blot extension of insight into the disrupted balance between angiogenic and antiangiogenic factors in the etiology of IUGR. The present data support the solid evidence of insufficient uteroplacental perfusion resulting in IUGR. The present authors further hypothesise that impaired expression of angiogenic factors can also, to some degree, represent a response to specific placental inflammation.

Acknowledgement

This work was supported by grant GAUK No. 1264214.

References

[1] Maynard S.E., Karumanchi S.A.: "Angiogenic factors and preeclampsia". *Semin. Nephrol.*, 2011, *31*, 33.
[2] Yancopoulos G.D., Davis S., Gale N.W., Rudge J.S., Wiegand S.J., Holash J.: "Vascular-specific growth factors and blood vessel formation". *Nature*, 2000, *407*, 242.
[3] Regnault T.R., Galan H.L., Parker T.A., Anthony R.V.: "Placental development in normal and compromised pregnancies— a review". *Placenta*, 2002, *23*, S119.
[4] Peleg D., Kennedy C.M., Hunter S.K.: "Intrauterine growth restriction: identification and management". *Am. Fam. Physician*, 1998, *58*, 453.
[5] Creasy R.K., Resnik R.: "Intrauterine growth restriction". *In*: Creasy R.K., Resnik R.,\ (eds). *Maternal-fetal medicine: principles and practice*. Philadelphia: Saunders, 1994, 558.
[6] Maulik D., Frances Evans J., Ragolia L.: "Fetal growth restriction: pathogenic mechanisms". *Clin Obstet Gynecol*, 2006, *49*(2), 219.
[7] Arroyo J.A., Winn V.D.: "Vasculogenesis and angiogenesis in the IUGR placenta". *Semin. Perinatol.*, 2008, *32*, 172.
[8] Ahmed A., Perkins J.: "Angiogenesis and intrauterine growth restriction". *Baillieres Best Pract. Res. Clin. Obstet. Gynaecol.*, 2000, *14*, 981.
[9] Seval Y., Sati L., Celik-Ozenci C., Taskin O., Demir R.: "The distribution of angiopoietin-1, angiopoietin-2 and their receptors tie-1 and tie-2 in the very early human placenta". *Placenta*, 2008, *29*, 809.
[10] Thurston G.: "Role of Angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lymphangiogenesis". *Cell Tissue Res.*, 2003, *314*, 61.
[11] Maisonpierre P.C., Suri C., Jones P.F., Bartunkova S., Wiegand S.J., Radziejewski C., *et al.*: "Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis". *Science*, 1997, *277*, 55.
[12] Davis S., Aldrich T.H., Jones P.F., Acheson A., Compton D.L., Jain V., *et al.*: "Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning". *Cell*, 1996, *87*, 1161.
[13] Saharinen P., Kerkela K., Ekman N., Marron M., Brindle N., Lee G.M., *et al.*: "Multiple angiopoietin recombinant proteins activate the Tie1 receptor tyrosine kinase and promote its interaction with Tie2". *J. Cell. Biol.*, 2005, *169*, 239.
[14] Suri C., McClain J., Thurston G., McDonald D.M., Zhou H., Oldmixon E.H., *et al.*: "Increased vascularization in mice overexpressing angiopoietin-1". *Science*, 1998, *282*, 468.
[15] Witzenbichler B., Maisonpierre P. C., Jones P., Yancopoulos G.D., Isner J.M.: "Chemotactic properties of angiopoietin-1 and -2, ligands for the endothelial-specific receptor tyrosine kinase Tie2". *J. Biol. Chem.*, 1998, *273*, 18514.
[16] Augustin H.G., Koh G.Y., Thurston G., Alitalo K.: "Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system". *Nat. Rev. Mol. Cell. Biol.*, 2009, *10*, 165.
[17] Thomas M., Augustin H.G.: "The role of the Angiopoietins in vascular morphogenesis". *Angiogenesis*, 2009, *12*, 125.
[18] Ng Y.S., Krilleke D., Shima D.T.: "VEGF function in vascular pathogenesis". *Exp. Cell. Res.*, 2006, *312*, 527.
[19] Frater J.L., Kay N.E., Goolsby C.L., Crawford S.E., Dewald G.W., Peterson L.C.: "Dysregulated angiogenesis in B-chronic lymphocytic leukemia: morphologic, immunohistochemical, and flow cytometric evidence". *Diagn. Patho.l*, 2008, *3*, 16.
[20] Shiraishi S., Nakagawa K., Kinukawa N., Nakano H., Sueishi K.: "Immunohistochemical localization of vascular endothelial growth factor in the human placenta". *Placenta*, 1996, *17*, 111.
[21] Carmeliet P.: "Angiogenesis in health and disease". *Nat. Med.*, 2003, *9*, 653.
[22] Rundhaug J.E.: "Matrix metalloproteinases and angiogenesis". *J. Cell. Mol. Med.*, 2005, *9*, 267.
[23] Zhu J.Y., Pang Z.J., Yu Y.H.: "Regulation of trophoblast invasion: the role of matrix metalloproteinases". *Rev. Obstet. Gynecol.*, 2012, *5*, e137.
[24] Bjorn S.F., Hastrup N., Larsen J.F., Lund L.R., Pyke C.: "Messenger RNA for membrane-type 2 matrix metalloproteinase, MT2-MMP, is

expressed in human placenta of first trimester". *Placenta*, 2000, *21*, 170.

[25] McKirdy A., Marks L.: "PP060. Matrix metalloproteinases-2 and -9 and their inhibitors: A role in the development of pre-eclampsia?" *Pregnancy Hypertens.*, 2012, *2*, 274.

[26] Huisman M. .A., Timmer A., Zeinstra M., Serlier E.K., Hanemaaijer R., Goor H., Erwich J.J.: "Matrix-metalloproteinase activity in first trimester placental bed biopsies in further complicated and uncomplicated pregnancies". *Placenta*, 2004, *25*, 253.

[27] Merchant S.J., Crocker I.P., Baker P.N., Tansinda D., Davidge S.T., Guilbert L.J.: "Matrix metalloproteinase release from placental explants of pregnancies complicated by intrauterine growth restriction". *J. Soc. Gynecol. Investig.*, 2004, *11*, 97.

[28] Geva E., Ginzinger D.G., Zaloudek C.J., Moore D.H., Byrne A., Jaffe R.B.: "Human placental vascular development: vasculogenic and angiogenic (branching and nonbranching) transformation is regulated by vascular endothelial growth factor-A, angiopoietin-1, and angiopoietin-2". *J. Clin. Endocrinol. Metab.*, 2002, *87*, 4213.

[29] Kappou D., Sifakis S., Androutsopoulos V., Konstantinidou A., Spandidos D.A., Papantoniou N.: "Placental mRNA expression of angiopoietins (Ang)-1, Ang-2 and their receptor Tie-2 is altered in pregnancies complicated by preeclampsia". *Placenta*, 2014, *35*, 718.

[30] Semczuk M., Borczynska A., Bialas M., Rozwadowska N., Semczuk-Sikora A., Malcher A., Kurpisz M.: "Expression of genes coding for proangiogenic factors and their receptors in human placenta complicated by preeclampsia and intrauterine growth restriction". *Reprod. Biol.*, 2013, *13*, 133.

[31] Vuorela P., Halmesmaki E.: "Vascular endothelial growth factor, its receptors, and the tie receptors in the placental bed of women with preeclampsia, diabetes, and intrauterine growth retardation". *Am. J. Perinatol.*, 2006, *23*, 255.

[32] Rajakumar A., Jeyabalan A., Markovic N., Ness R., Gilmour C., Conrad K.P.: "Placental HIF-1 alpha, HIF-2 alpha, membrane and soluble VEGF receptor-1 proteins are not increased in normotensive pregnancies complicated by late-onset intrauterine growth restriction". *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2007, *293*, R766.

[33] Helske S., Vuorela P., Carpen O., Hornig C., Weich H., Halmesmaki E.: "Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies". *Mol. Hum. Reprod.*, 2001, *7*, 205.

[34] Andraweera P.H., Dekker G.A., Laurence J.A., Roberts C.T.: "Placental expression of VEGF family mRNA in adverse pregnancy outcomes". *Placenta*, 2012, *33*, 467.

[35] Zhou Y., McMaster M., Woo K., Janatpour M., Perry J., Karpanen T., *et al.*: "Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome". *Am. J. Pathol.*, 2002, *160*, 1405.

[36] Nevo O., Lee D.K., Caniggia I.: "Attenuation of VEGFR-2 expression by sFlt-1 and low oxygen in human placenta". *PLoS One*, 2013, *8*, e81176.

[37] Pascal L.E., True L.D., Campbell D.S., Deutsch E.W., Risk M., Coleman I.M., *et al.*: "Correlation of mRNA and protein levels: cell type-specific gene expression of cluster designation antigens in the prostate". *BMC Genomics*, 2008, *9*, 246.

[38] Bolin M., Wiberg-Itzel E., Wikstrom A.K., Goop M., Larsson A., Olovsson M., Akerud H.: "Angiopoietin-1/angiopoietin-2 ratio for prediction of preeclampsia". *Am. J. Hypertens.*, 2009, *22*, 891.

[39] Koutroubakis I.E., Xidakis C., Karmiris K., Sfiridaki A., Kandidaki E., Kouroumalis E.A.: "Potential role of soluble angiopoietin-2 and Tie-2 in patients with inflammatory bowel disease". *Eur. J. Clin. Invest.*, 2006, *36*, 127.

[40] Fiedler U., Augustin H.G.: "Angiopoietins: a link between angiogenesis and inflammation". *Trends Immunol.*, 2006, *27*, 552.

[41] Inoue H., Nishio H., Takada H., Sakai Y., Nanishi E., Ochiai M., *et al.*: "Activation of Nod1 signaling induces fetal growth restriction and death through fetal and maternal vasculopathy". *J. Immunol.*, 2016, *196*, 2779.

[42] Zhu J., Zhong M., Pang Z., Yu Y.: "Dysregulated expression of matrix metalloproteinases and their inhibitors may participate in the pathogenesis of pre-eclampsia and fetal growth restriction". *Early Hum. Dev.*, 2014, *90*, 657.

[43] Hu J., Van den Steen P.E., Sang Q.X., Opdenakker G.: "Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases". *Nat. Rev. Drug Discov.*, 2007, *6*, 480.

Corresponding Author:
I. SVANDOVA M.Sc.
Faculty of Science, Charles University
Vinicna, 7
Prague 2, 128 44 (Czech Republic)
e-mail: iva@natur.cuni.cz