Detyrosinated tubulin is decreased in fetal vessels of preeclampsia placentas

H. Huebner [a, b, *], B. Knoerr [b], A. Betzler [b], A. Hartner [c], S. Kehl [b], F. Baier [b], D.L. Wachter [d], R. Strick [b], M.W. Beckmann [b], F.B. Fahlbusch [c], M. Ruebner [b]

[a] *Institute for Women's Healthcare (IFG), Erlangen, Germany*
[b] *Department of Gynecology and Obstetrics, University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany*
[c] *Department of Pediatrics and Adolescent Medicine, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany*
[d] *Institute of Pathology, University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany*

1. Introduction

Preeclampsia (PE) still remains a major cause of maternal and fetal morbidity and mortality [1]. It is associated with maternal hypertension and proteinuria and often complicated by fetal growth restriction [1,2]. Furthermore, clinical complications like maternal liver or kidney malfunctions, thrombocytopenia or edemas are commonly detected.

Human placental development is highly dependent on the proliferation, migration and invasion of trophoblasts into the maternal decidua and myometrium in early pregnancy with consecutive transformation of spiral arteries supplying the intervillous space [3]. The establishment of a low pressure high-flow system allows sufficient flow to the developing villous circulation, which undergoes progressive arborization and vascularization until term [3]. Impairment of maternal spiral artery remodeling and fetal villi circulation is discussed to be a leading cause of PE [4–6]. On the one hand, remodeling of maternal spiral arteries is hindered during placental development of PE, leading to reduced placental perfusion and an insufficient oxygen and nutrient supply of the growing fetus [7]. On the other hand, fetal vessels show an altered

* Corresponding author. Department of Gynecology and Obstetrics, University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany.
E-mail address: Hanna.huebner@uk-erlangen.de (H. Huebner).

morphology with fewer branches and a more uncoiled structure [8,9].

The maintenance and morphology of an integral vascular system is highly reliant on an intact endothelial cytoskeleton [10]. It is well known that microtubule-targeting agents inhibit angiogenesis and vascularization through the disruption of the tubulin and actin cytoskeleton [11,12]. Microtubules (MT) are important cellular matrix structures, essential for processes like mitosis, intracellular transportation, cell motility and stability [13,14]. α- and β-tubulin are the major components of MTs. Through polymerization of α- and β-tubulin dimers linear protofilaments arise, which form the basic structure of one MT [15]. MT dynamics are influenced by posttranslational modifications of α- and β-tubulin. These can alter the binding capacity of tubulin to other proteins and influence the MT stabilization and depolymerization [13]. Such modifications are tyrosination, detyrosination, acetylation and polyglutamylation of α-tubulin as well as phosphorylation and polyglycylation of β-tubulin [16]. Most of all, the reversible tyrosination/deytrosination of α-tubulin is discussed to be of impact for the stability and dynamicity of MTs. Especially the detyrosinated form of α-tubulin is associated with MT stability and is important for epithelial-to-mesenchymal transition [17].

However, little is known about the expression pattern of detyrosinated (detyr) and, its counterpart, tyrosinated (tyr) α-tubulin in healthy and diseased placentas. Thus, we set out to investigate the specific expression in placental tissues and analyzed the dysregulation in placentas complicated by PE and small of gestational age (SGA).

2. Material and methods

2.1. Patient and tissue collection

Human placentas were obtained from healthy pregnancies (n = 21) or pregnancies complicated by PE and/or SGA (n = 5, each). After removal of the basal plate and chorionic membrane, a biopsy was obtained about 3 cm distal from the cord of every placenta. The clinical data of all patients are summarized in Table 1. The inclusion criteria for PE and SGA were described earlier by Fahlbusch and Ruebner et al. [18,19]. SGA was defined as ≤10th percentile of weight for gestational age. For immunohistochemical and immunofluorescence staining, tissues were fixed in formaldehyde and embedded in paraffin (FFPE). For protein extraction biopsies were taken following a standardized protocol and snap frozen in liquid nitrogen within one hour after delivery, as previously described [20].

2.2. Ethical approval

Each participant signed a written informed consent. Handling of patients and tissues was approved by the Ethics Committee at the University of Erlangen-Nuremberg (No. 353_15B).

2.3. Immunohistochemical and immunofluorescence staining

Immunohistochemistry was performed using the LSAB + HRP kit (Agilent, Hamburg, Germany) according to the manufacturer's instructions. Immunofluorescence staining was performed as described earlier [21]. Mouse anti-Human tyr-tubulin monoclonal antibody (1:500 for immunohistochemistry and 1:800 for immunofluorescence, T9028, Sigma-Aldrich, Taufkirchen, Germany), Rabbit anti-human detyr-tubulin polyclonal antibody (1:500 for immunohistochemistry and 1:200 for immunofluorescence, ab48389, Abcam, Cambridge, UK), Mouse anti-human smooth muscle actin (SMA) monoclonal antibody (1:100, #MO851, Dako, Agilent, Hamburg, Germany), Mouse anti-human CD31 monoclonal antibody (1:100, ab199012, Abcam, Cambridge, UK), Alexa Fluor 488 Donkey anti-mouse IgG (H + L) (1:1000, A-21202, Thermo Fisher, Darmstadt, Germany) and Alexa Fluor 594 Donkey anti-rabbit IgG (H + L) (1:1000, A-21207, Thermo Fisher, Darmstadt, Germany) were used. Nuclei were counter-stained with hematoxylin or Hoechst 33342. Negative controls for immunohistochemistry and immunofluorescence were performed using the respective secondary antibodies, only. Protein localization of detyr-tubulin was further analyzed on an organ tissue microarray (TMA) by immunohistochemistry. Stained TMA slides were digitalized using the Pannoramic 250 Flash II Scanner and the Case Viewer 2.1 software package (both 3DHistech, Hungary).

2.4. Isolation of primary trophoblasts

Primary trophoblasts were isolated from four healthy placentas using the Trypsin-DNase-Dispase/Percoll method, as previously described [23].

2.5. Protein extraction

Proteins were extracted from freshly frozen biopsies using a microdismembranator and suspended in RIPA buffer (Sigma Aldrich, Taufkirchen, Germany) with 1 mM Na3VO4, 1.5 mM NaF and protein inhibitor cocktail (1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/mL of each pepstatin, leupeptin and chymostatin). Protein lysates from primary trophoblasts were obtained through lysis with RIPA buffer as stated above. Protein concentrations were measured using the EZQ protein quantitation kit (Thermo Fisher, Darmstadt, Germany).

2.6. Western blot analysis

20 or 40 μg of each lysate was resolved on a 10% acrylamide-SDS gel and transferred to a nitrocellulose membrane using Tris−glycin buffer. Blocking was performed with 5% nonfat milk. Mouse anti-human tyr-tubulin monoclonal antibody (1:5000, T9028, Sigma-Aldrich, Taufkirchen, Germany), rabbit anti-human detyr-tubulin polyclonal antibody (1:500, ab48389, Abcam, Cambridge, UK), donkey anti-mouse IgG-HRP antibody (1:20,000, AP192P, Merck Millipore, Billerica, Massachusetts, USA), goat anti-rabbit IgG-HRP antibody (1:1,000, #7074, Cell Signaling, Cambridge, UK), mouse anti-human CD31 monoclonal antibody (1:400, ab199012, Abcam, Cambridge, UK) and rabbit anti-human GAPDH polyclonal IgG-HRP antibody (1:1,000, sc-25778, Santa Cruz, Heidelberg, Germany) were used.

2.7. Statistical analysis

Western blot bands were quantified using ImageJ (NIH, [24]) All data are presented as mean ± standard error of the mean (SEM). Differences were analyzed using the Mann-Whitney U-test (SPSS, IBM Inc., Ehningen, Germany). P-values of ≤ .05 were considered as statistically significant.

3. Results

3.1. Detyr-tubulin occurrence in trophoblast cell lines and placental compartments

The amount of detyr-tubulin in placental tissue and primary trophoblasts was analyzed by Western Blot (Fig. 1 **A**). Strong detyr-tubulin signals were detected in placental tissue, with no detectable expression in primary villous trophoblasts (Fig. 1 **A**). In

Table 1
Clinical data.

Parameter/Cohort	Control (n = 21)	SGA (n = 5)	PE/SGA (n = 5)	PE (n = 5)
Gravidity (Median, 25–75%)	2 (2–3)	3 (2–3)	1 (1–2)	1 (1–2)
Parity (Median, 25–75%)	1 (0–1)	1 (0–2)	0 (0–0)	0 (0–0)
Gestational age, weeks (Mean ± SD)	37.6 ± 1.96	37.2 ± 2.71	37.4 ± 1.85	37.2 ± 1.47
Maternal age, years (Mean ± SD)	33.1 ± 3.93	33.8 ± 3.71	29.4 ± 3.14	31.4 ± 3.93
Race	White (n = 20), Black (None), others (n = 1)	White (n = 5), Black (None)	White (n = 5), Black (None)	White (n = 5), Black (None)
Ethnicity	German (n = 17), Bosniak (n = 1), Philippine(n = 1), Russian (n = 1)	German (n = 4), Turkish (n = 1)	German (n = 4), Polish (n = 1)	German (n = 5)
Prenatal medications	Iron (n = 1), L-Thyroxin (n = 1), Magnesium (n = 1), Tachyferon (n = 1)	Iron (n = 1)	None	None
Drugs (alcohol, tabacco or other drugs)	None	None	None	None
Blood pressures < 140/90 mm Hg	Yes (n = 21)	Yes (n = 5)	No (n = 5)	No (n = 5)
Antibiotics in labor	None (n = 7), Penicillin (n = 14),	None (n = 2), Penicillin (n = 3)	None (n = 2), Penicillin (n = 3)	None (n = 1), Penicillin (n = 4)
Beta strep status	Positive (n = 1), negative (n = 10), unknown (n = 10)	Negative (n = 2), Unknown (n = 3)	Negative (n = 2), Unknown (n = 3)	Negative (n = 1), Unknown (n = 4)
Delivery mode	C-section repeat (n = 5), C-section primary (n = 7), Spontaneous (n = 9)	C-section repeat (n = 1), C-section primary (n = 2), Spontaneous (n = 2)	C-sectio repeat (n = 2), C-section primary (n = 1), Spontaneous (n = 2)	C-sectio repeat (n = 1), C-sectio primary (n = 2), Spontaneous (n = 2)
Birth weight, grams (mean ± SD)	3190 ± 589	2130 ± 579	2241 ± 278	2778 ± 641
Percentile at birth (Median, 25–75%)	49 (29–65)	8 (2–10)	4 (4–7)	29 (15–48)
Baby's sex	Female (n = 8), Male (n = 13)	Female (n = 2), Male (n = 3)	Female (n = 3), Male (n = 2)	Female (n = 3), Male (n = 2)

contrast, Tyr-tubulin expression was present in both placental tissue and primary trophoblasts (Fig. 1 **A**). In order to investigate the specific placental expression pattern of detyr-tubulin, we performed immunohistochemistry (Fig. 1 **B, a-f**) and immunofluorescence (Fig. 1 **B, g-i**) staining of placental FFPE sections. We analyzed the expression pattern in first trimester (Fig. 1 B **a-c)** and third trimester placental tissue (Fig. 1 B **d-i**). A strong detyr-tubulin expression was detected within the fetal part of the human placenta, which was in particular localized around the fetal vascular structures (Fig. 1 **B, b and e black arrow heads**). In contrast, maternal vessels had a considerable weaker detyr-tubulin staining pattern in both first and third trimester tissue (Fig. 1 **B, c, f and i asterisk**). The high expression of detyr-tubulin in close proximity to fetal vessels was confirmed by immunofluorescence staining of third trimester placental tissue (Fig. 1 **B, g and h**). Human villous and extravillous trophoblasts were negative for detyr-tubulin (Fig. 1 **B**), while we observed some positive staining for detyr-tubulin in decidual stroma cells (Fig. 1 **B, f and i white arrow heads**).

3.2. Expression of detyr-tubulin in fetal endothelial cells

Co-immunofluorescence staining of placental FFPE sections with anti-detyr-tubulin and anti-CD31 antibodies showed a specific expression of detyr-tubulin in fetal endothelial cells (Fig. 2). The anti-CD31 antibody was used as an established marker for endothelial cells. A co-localization of detyr-tubulin and CD31 could specifically be shown in fetal endothelial cells (Fig. 2 **A, g and h**), while maternal endothelial cells were negative for detyr-tubulin (Fig. 2 **A, i**). In order to analyze whether vascular smooth muscle cells were positive for detyr-tubulin, we performed a co-immunofluorescence staining with smooth muscle actin (SMA) (Fig. 2 **B**). Fetal vascular smooth muscle cells showed a strong SMA expression (Fig. 2 **B, d and e**), but no co-localization with detyr-tubulin (Fig. 2 **B, g and h**). We further analyzed whether this vascular pattern was specific for placental tissue by immunhistochemical and immunofluorescence staining of an organ tissue micro array (TMA) (Supplementary Fig. 1). The vessels of the fetal placental tissue showed the strongest detyr-tubulin staining of all endothelial cells.

3.3. Localization of detyr- and tyr-tubulin

In order to investigate, whether the high vascular expression of detyr-tubulin is linked to a reduced presence of the tyrosinated form, we further analyzed the co-localization of detyr- and tyr-tubulin via co-immunofluorescence staining of placental sections (Fig. 3). In contrast to detyr-tubulin, tyr-tubulin showed a strong expression pattern in villous and extravillous trophoblasts as well as in villous stroma cells (Fig. 3 **d-f, arrow heads**). We could detect only a sparse co-localization of detyr- and tyr-tubulin on the basal side of the endothelium (Fig. 3 **h, ▷ arrow head**).

3.4. Reduced detyr-tubulin expression in placental pathologies

An impairment of placental angiogenesis and vascularization is often discussed to be a leading cause of PE, which is often associated with a reduced fetal growth. Thus, we were interested in the expression pattern and amount of detyr-tubulin in placentas complicated by PE and/or SGA. Immunohistochemical staining of placental sections from healthy controls, SGA and PE cases, showed a weaker detyr-tubulin staining intensity in endothelial cells from pathologies compared to controls (Fig. 4 **A**). In order to quantify this expression, we extracted protein from 21 healthy placentas, 5 SGA, 5 SGA/PE and 5 PE cases and performed western blot analysis of

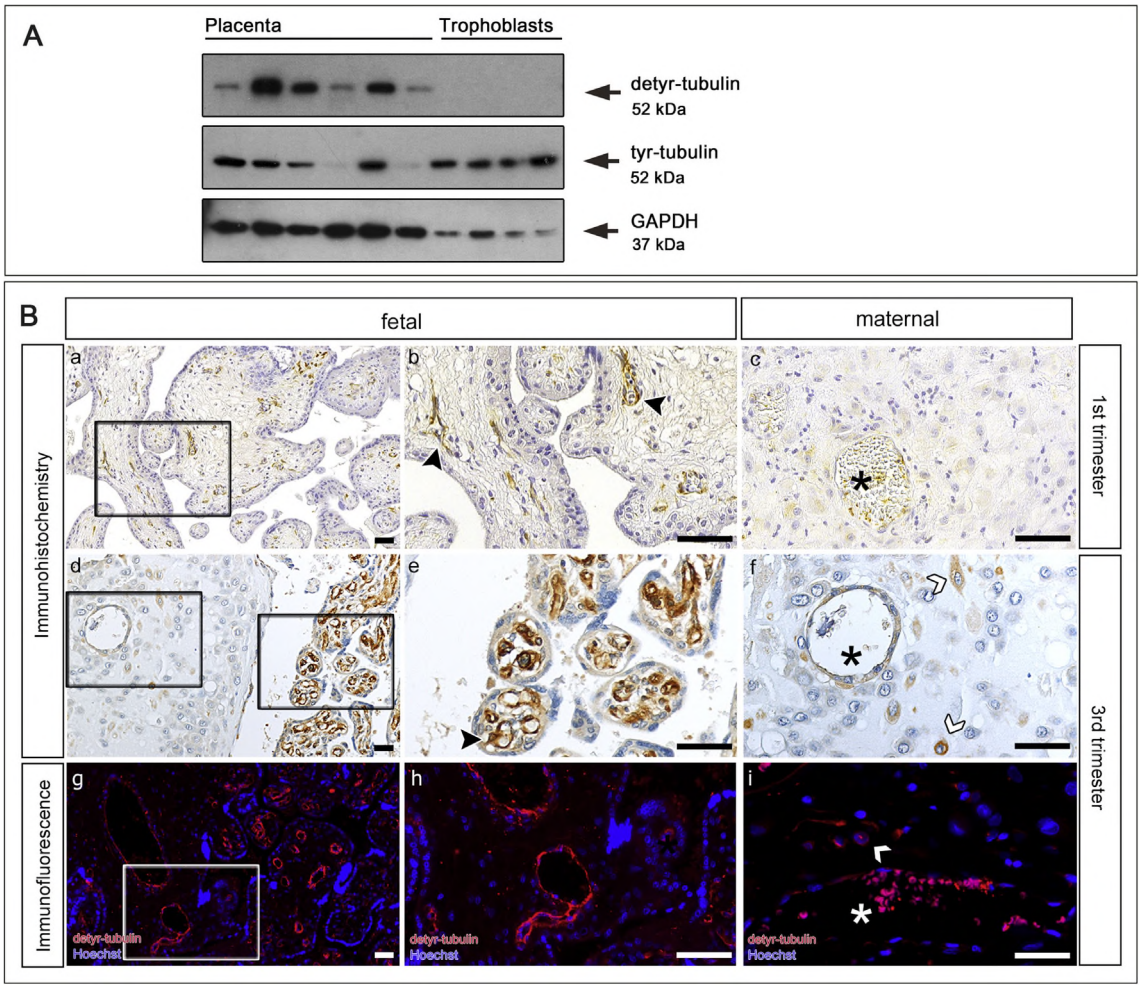

Fig. 1. **A**) Protein expression of detyr-tubulin (52 kDa), tyr-tubulin (52 kDa) and the housekeeping gene GAPDH (37 kDa) was visualized by Western blot. Protein lysates (40 μg) from six placental biopsies and four primary trophoblast isolates were used. **B**) The expression pattern of detyr-tubulin in human FFPE placental tissues from the first trimester (a–c) and third trimester (d–i) was analyzed by immunohistochemistry (a–f) and immunofluorescence (g–i) staining. b, e, f and h are magnifications of the areas marked by rectangles in a, d and g. Black and white arrow heads mark detyr-tubulin positive endothelial cells or decidual stroma cells, respectively. The asterisks mark maternal vessels. The bar equals 100 μm.

detyr-tubulin, tyr-tubulin and CD31 (Fig. 4 **B, a**). Western blot bands were quantified using ImageJ and the mean of all control samples ($n = 21$) was compared to the mean intensity of each pathologic cohort ($n = 5$, respectively) (Fig. 4 **B, b**). We detected a significantly reduced amount of detyr-tubulin in PE/SGA and PE cases compared to healthy controls (Fig. 4 **B, b**, $p = .028$ and .005, respectively). Additionally, western blot bands of tyr-tubulin were quantified. No significant differences of tyr-tubulin were detected when comparing healthy controls with PE/SGA or PE cases (Fig. 4 **B, b**). In order to investigate, whether the endothelial cells content decreased in placentas of PE and SGA cases, we additionally analyzed CD31 expression. A significantly reduced CD31 expression could only be detected in PE/SGA placental tissues (Fig. 4 **B, b**).

4. Discussion

In summary, we detected a strong presence of detyr-tubulin in the fetal endothelial cells of the healthy human placenta. In contrast, placentas complicated by PE had a significantly reduced amount of detyr-tubulin. In general, tyrosination and detyrosination of α-tubulin are important for the plasticity of microtubules. High detyr-tubulin was associated with epithelial-to-mesenchymal transition (EMT) during metastasis and re-adhesion of breast cancer cells [17]. During this process most of the detyr-tubulin expression could be detected within the breast cancer cell microtentacles [17]. Interestingly, during early placental development EMT occurs mainly in extravillous trophoblasts, which detach from the anchoring villi and invade into the maternal decidua [25]. Here we showed that third trimester extravillous trophoblasts were detyr-tubulin negative and that the placental accumulation of detyr-tubulin was mainly within fetal endothelial cells. We assume that this might be due to the advanced stage of placental samples used. All placental biopsies were taken at term, with a mean gestational age of 37.5 weeks, which indicates a reduced invasiveness and advanced differentiation of extravillous trophoblasts. On the other hand, increased detyrosination of α-tubulin was associated with stable microtubules and vascular tube morphogenesis during angiogenesis of endothelial cells [26,27]. Detyr-tubulin seemed to be involved in vascular tube morphogenesis by increasing vascular tube lumen [28]. Additionally, detyr-tubulin was discussed to be essential for the recruitment and binding of motor proteins, like Kinesin-1 [29]. Vascular endothelial growth factor receptor -2 and -3 are able to bind to Kinesin-1, which leads to their transportation to the surface of endothelial cells and the

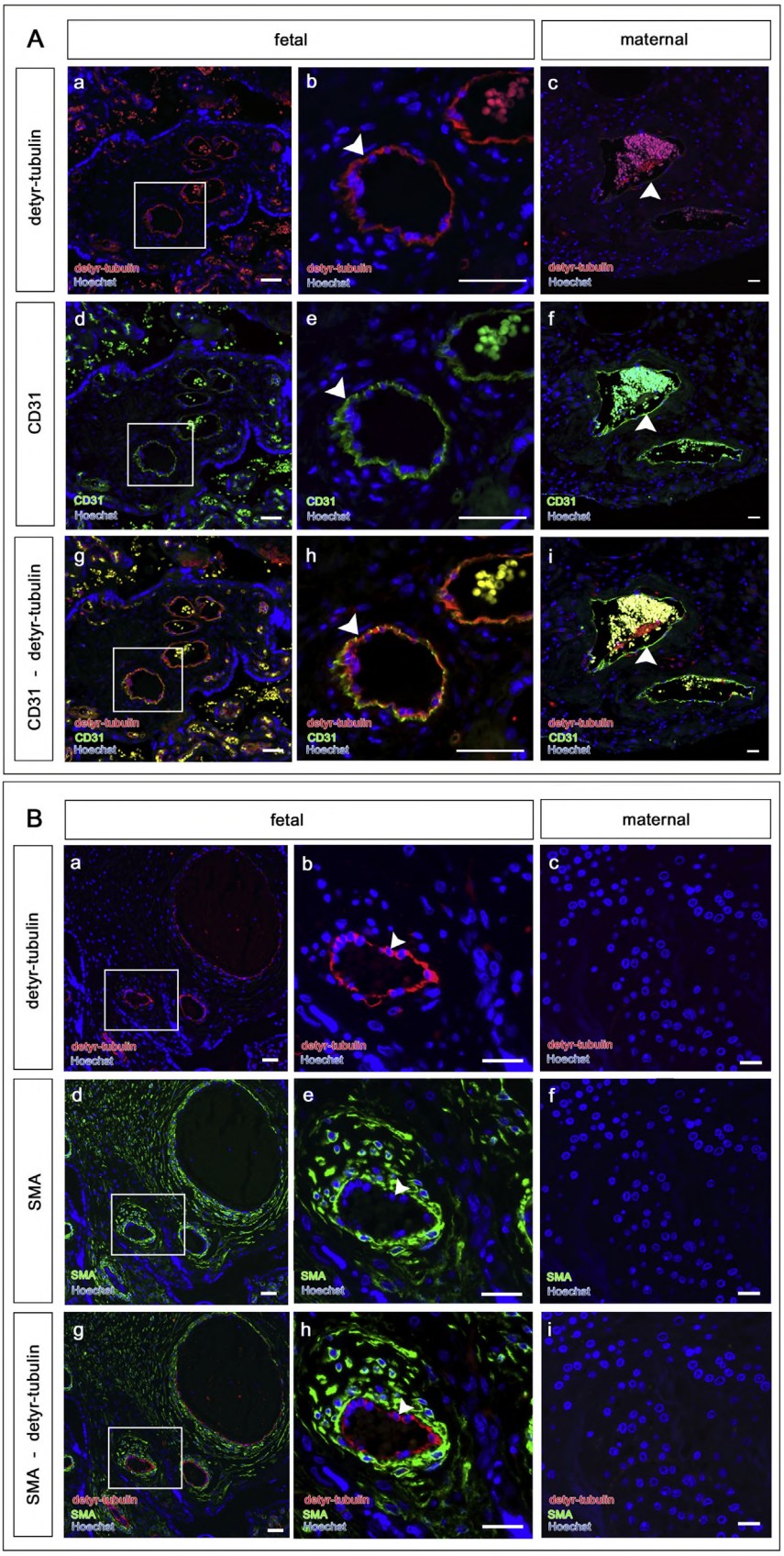

Fig. 2. **A**) Placental FFPE sections were incubated with anti-detyr-tubulin (a–c) and anti-CD31 (d–f) antibodies and stained using immunofluorescence. Detyr-tubulin and CD31 positive endothelial cells are marked by white arrow heads. The merge signals are shown in g-i. Nuclei were stained with Hoechst 33342. White arrow heads mark detyr-tubulin positive (b, e and h) or negative (c, f and i) endothelial cells. Asterisk marks unspecific staining of blood cells. **B**) Placental FFPE sections were incubated with anti-detyr-tubulin (a–c) and anti-smooth muscle actin (SMA) (d–f) antibodies and stained using immunofluorescence. White arrow heads mark detyr-tubulin positive endothelial cells (b, e and h). Merged images are shown in g-i. The bars equal 100 μm.

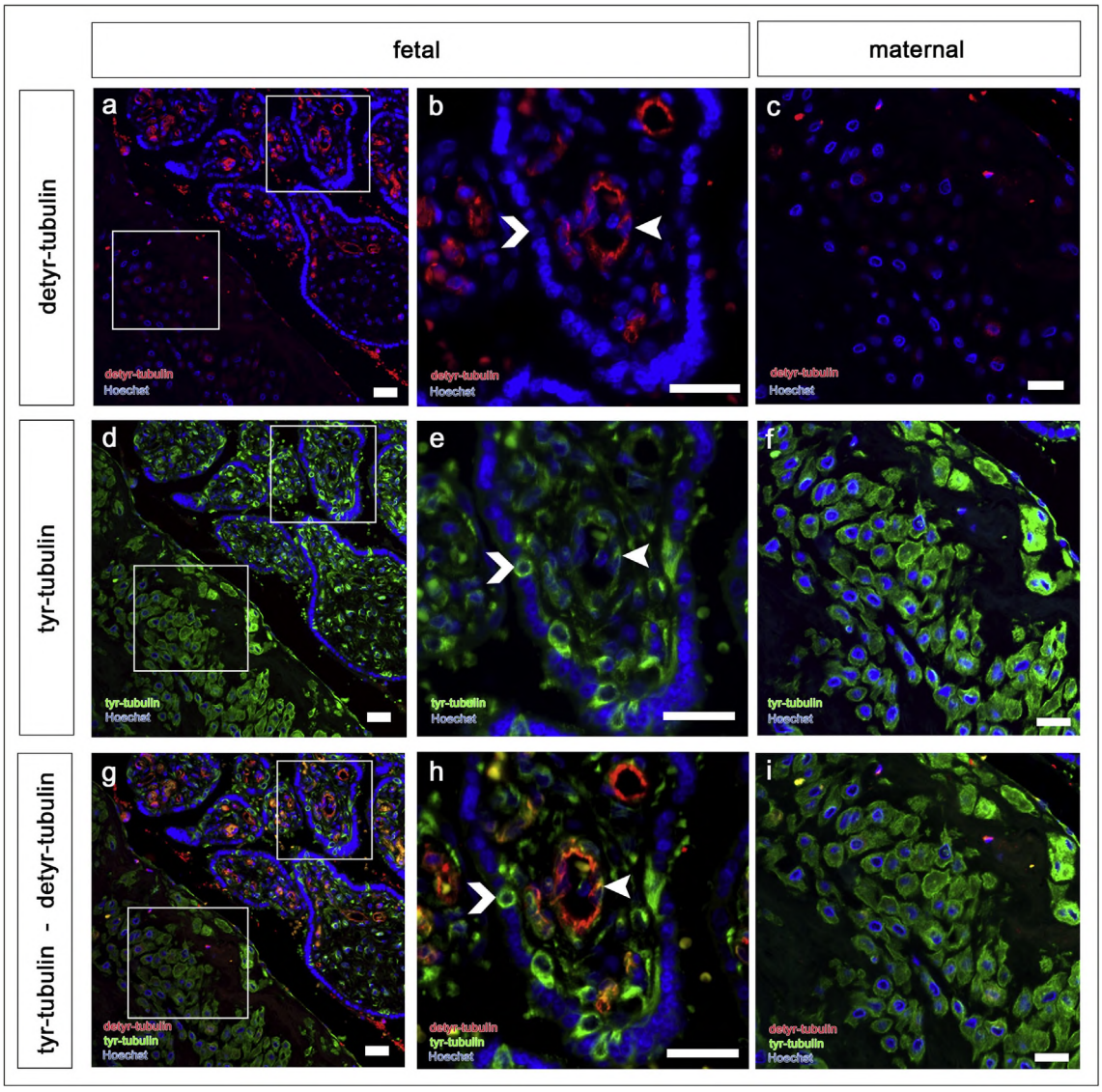

Fig. 3. Placental FFPE sections were incubated with anti-detyr-tubulin (a–c) and anti-tyr-tubulin (d–f) antibodies. Tyr-tubulin positive cells are marked by white arrow heads (≫ for villous trophoblasts and ▷ for endothelial cells). Merged images are shown in g-i. Nuclei were stained with Hoechst 33342. The bars equal 100 μm.

induction of invasion and migration [30]. During placental development the villous vascular system is established in two major steps. First, vasculogenesis allows the formation of primary endothelial cells by differentiation of pluripotent mesenchymal cells [31]. Second, new vessels and branches arise through angiogenesis [31]. Thus, we hypothesize that the high expression of detyr-tubulin in placental endothelial cells might be due to an ongoing placental angiogenesis and points out that detyr-tubulin might be of importance for the physiologic angiogenesis of the villous tree. This goes in line with the detected decrease of detyr-tubulin in placentas complicated by PE. It is well known that PE is a pathologic condition which arises from an abnormal placental development [1]. Decreased expression of Syncytin-1, following impaired villous trophoblast fusion, and increased apoptosis of trophoblasts are associated with PE [23,32]. The fetal histopathology of PE is characterized by an impaired villous vascular system with elongated, poorly branched and capillarized vessels, which can clinically be used for screening and monitoring of PE via 3D power Doppler angiography in the first and second trimester [3,33]. While maternal artery remodeling is discussed as the leading etiopathologic cause of PE, changes in the feto-placental vascular system are still common in PE placenta. The latter alterations are commonly associated with an increased long term cardiovascular risk for the offspring and may induce fetal growth restriction [33,34].

Increased levels of anti-angiogenic factors, like soluble fms-related tyrosine kinase 1(sFlt-1), soluble endoglin or soluble vascular endothelial growth factor receptor 1 were associated with PE and were reported to be responsible for the inhibition of placental angiogenesis [6,35–37]. This underscores the importance of vascularization and angiogenesis for human placental development and pregnancy. Here, we detected only a slight decrease of CD31 expression in PE samples. This might be due to the sampling procedure as we removed the basal plate, and thus most of the decidua vascular system, before protein extraction. We assume that the reduced vascularization mainly concerns maternal vessels but not fetal ones.

By using an organ TMA we also compared the placental endothelial-specific expression of detyr-tubulin with its expression pattern in other organs. We detected a strong vascular expression

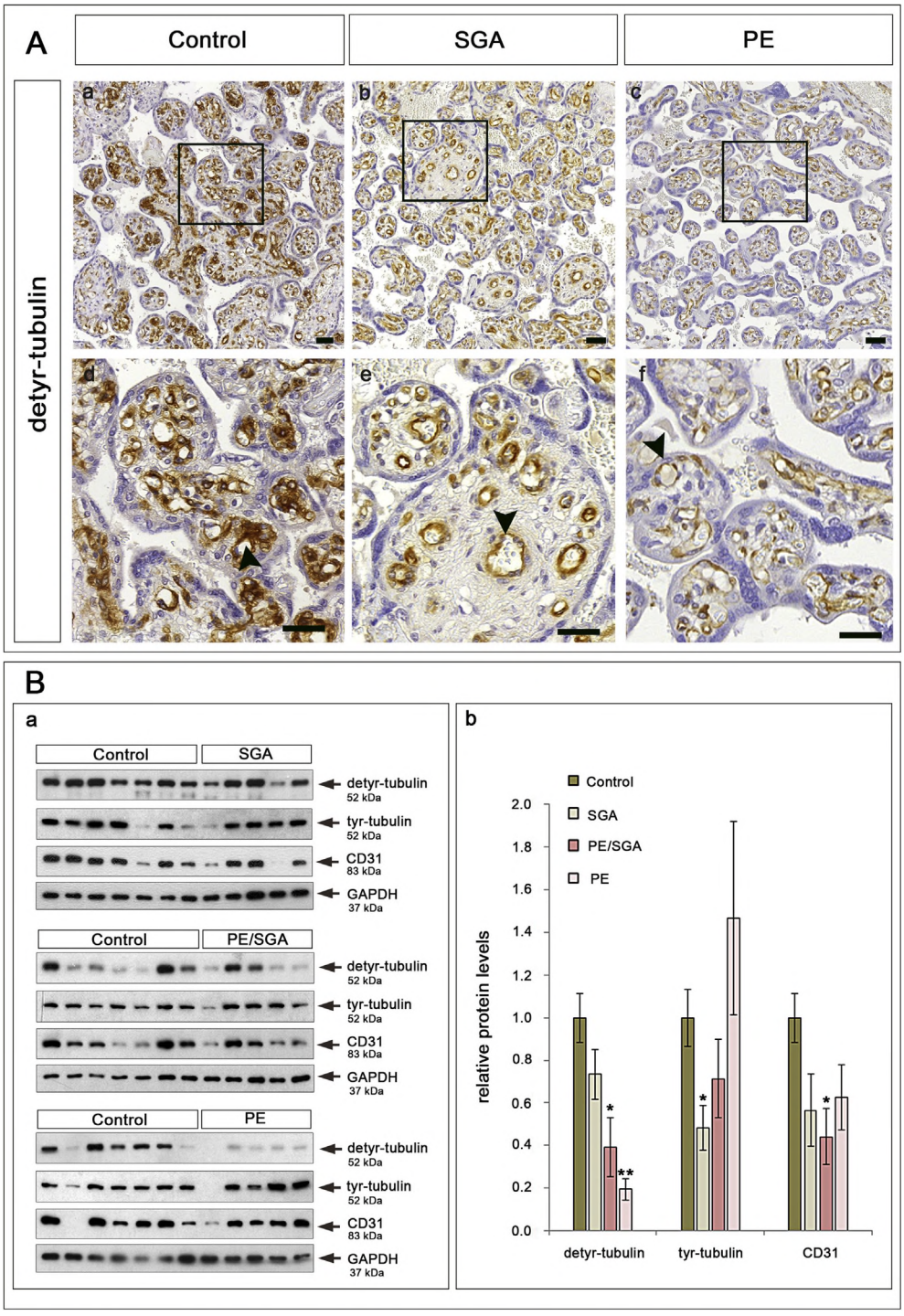

Fig. 4. **A**) Detyr-tubulin expression was analyzed by immunohistochemistry on healthy controls (a, d) and placentas from pregnancies complicated by SGA (b, e) or PE (c, f). Black arrow heads mark detyr-tubulin positive endothelial cells. The bars equal 50 μm. **B**) Detyr-tubulin, tyr-tubulin, CD31 and GAPDH expression in protein lysates from healthy (n = 21) and pathologic (n = 5, SGA, PE/SGA and PE) placentas were analyzed by Western blot (a). The intensities of detyr-tubulin, tyr-tubulin and CD31 were quantified using ImageJ and normalized to the according GAPDH intensity. The mean normalized intensity values of all controls (n = 21) and the pathologic cases (n = 5, respectively) ± the standard error of the mean are shown in b. The mean intensities of the healthy controls were set to 1. Significances were calculated using the Mann-Whitney-U test and p-values relative to the controls are indicated by asterisk with * $p < .05$ and ** $p < .005$.

only in placental tissue, while other organs showed high detyr-tubulin staining in other than endothelial cells. We assume that this might be due to the lower activity of angiogenesis in fully developed organs [38]. For further studies it might be of interest to block the tubulin detyrosination in fetal endothelial cells and to analyze tube formation and angiogenesis. In summary, we report a predominant localization of detyr-tubulin in fetal vessels of the placenta and a significantly reduced level of detyr-tubulin in placental biopsies of PE cases. Based on these findings, we hypothesize that detyrosination of α-tubulin is of importance for healthy placental vascularization and successful maintenance of pregnancy. Clinically, this could further underscore the role of 3D

power Doppler angiography monitoring of villous circulation during gestation to identify women at risk.

The value of our findings is limited by the following aspects: First, we did not analyze the expression of angiogenesis-related factors, which are known to be differentially expressed in sera of women developing PE (e.g. placental growth factor PlGF or sFlt-1). A side-by-side comparison of feto-placental detyr-tubulin reduction and sFlt-1/PlGF ratio could help to further strengthen our hypothesis [39]. Second, a complete histologic evaluation of the placenta was not performed. Consequently, we were not able to correlate placental pathological findings associated with maternal and fetal malperfusion and the amount of detected placental detyr-tubulin [40]. For future studies, an in-depth investigation of detyr-tubulin expression, placental histologic assessment and the correlation with angiogenesis-related factors would be of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.placenta.2017.12.019.

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References

[1] B. Sibai, G. Dekker, M. Kupferminc, Pre-eclampsia, Lancet 365 (9461) (2005) 785–799.
[2] R.B. Ness, B.M. Sibai, Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia, Am. J. Obstet. Gynecol. 195 (1) (2006) 40–49.
[3] S. Campbell, Placental vasculature as visualized by 3D power Doppler angiography and 3D color Doppler imaging, Ultrasound Obstet. Gynecol. 30 (6) (2007) 917–920.
[4] L. Brodowski, J. Burlakov, S. Hass, C. von Kaisenberg, F. von Versen-Höynck, Impaired functional capacity of fetal endothelial cells in preeclampsia, PLoS One 12 (5) (2017) e0178340.
[5] J.M. Roberts, R.N. Taylor, T.J. Musci, G.M. Rodgers, C.A. Hubel, M.K. McLaughlin, Preeclampsia: an endothelial cell disorder, Am. J. Obstet. Gynecol. 161 (5) (1989) 1200–1204.
[6] S. Ahmad, A. Ahmed, Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia, Circ. Res. 95 (9) (2004) 884–891.
[7] C.W. Redman, I.L. Sargent, Latest advances in understanding preeclampsia, Science (New York, NY) 308 (5728) (2005) 1592–1594.
[8] M. Zygmunt, F. Herr, K. Münstedt, U. Lang, O.D. Liang, Angiogenesis and vasculogenesis in pregnancy, Eur. J. Obstet. Gynecol. Reprod. Biol. 110 (2003) S10–S18.
[9] M.R. Jackson, A.J. Walsh, R.J. Morrow, B.M. Muller, S.J. Lye, J.K. Ritchie, Reduced placental villous tree elaboration in small-for-gestational-age pregnancies: relationship with umbilical artery Doppler waveforms, Am. J. Obstet. Gynecol. 172 (2) (1995) 518–525.
[10] K.J. Bayless, G.E. Davis, Microtubule depolymerization rapidly collapses capillary tube networks in vitro and angiogenic vessels in vivo through the small GTPase Rho, J. Biol. Chem. 279 (12) (2004) 11686–11695.
[11] M.N. Bijman, G.P. van Nieuw Amerongen, N. Laurens, V.W. van Hinsbergh, E. Boven, Microtubule-targeting agents inhibit angiogenesis at subtoxic concentrations, a process associated with inhibition of Rac1 and Cdc42 activity and changes in the endothelial cytoskeleton, Mol. Canc. Therapeut. 5 (9) (2006) 2348–2357.
[12] E. Pasquier, N. Andre, D. Braguer, Targeting microtubules to inhibit angiogenesis and disrupt tumour vasculature: implications for cancer treatment, Curr. Cancer Drug Targets 7 (6) (2007) 566–581.
[13] C.P. Garnham, A. Roll-Mecak, The chemical complexity of cellular microtubules: tubulin post-translational modification enzymes and their roles in tuning microtubule functions, Cytoskeleton 69 (7) (2012) 442–463.
[14] D. Wloga, J. Gaertig, Post-translational modifications of microtubules, J. Cell Sci. 123 (20) (2010) 3447–3455.
[15] R.C. Weisenberg, Microtubule formation in vitro in solutions containing low calcium concentrations, Science (New York, NY) 177 (4054) (1972) 1104–1105.
[16] M.M. Magiera, C. Janke, Post-translational modifications of tubulin, Curr. Biol. 24 (9) (2014) R351–R354.
[17] R.A. Whipple, M.A. Matrone, E.H. Cho, E.M. Balzer, M.I. Vitolo, J.R. Yoon, O.B. Ioffe, K.C. Tuttle, J. Yang, S.S. Martin, Epithelial-to-mesenchymal transition promotes tubulin detyrosination and microtentacles that enhance endothelial engagement, Canc. Res. 70 (20) (2010) 8127–8137.
[18] F.B. Fahlbusch, Y. Dawood, A. Hartner, C. Menendez-Castro, S.C. Nögel, A. Tzschoppe, H. Schneider, P. Strissel, M.W. Beckmann, E. Schleussner, Cullin 7 and Fbxw 8 expression in trophoblastic cells is regulated via oxygen tension: implications for intrauterine growth restriction? J. Matern. Fetal Neonatal Med. 25 (11) (2012) 2209–2215.
[19] M. Ruebner, P.L. Strissel, A.B. Ekici, E. Stiegler, U. Dammer, T.W. Goecke, F. Faschingbauer, F.B. Fahlbusch, M.W. Beckmann, R. Strick, Reduced syncytin-1 expression levels in placental syndromes correlates with epigenetic hypermethylation of the ERVW-1 promoter region, PLoS One 8 (2) (2013) e56145.
[20] K. Heussner, M. Ruebner, H. Huebner, W. Rascher, C. Menendez-Castro, A. Hartner, F.B. Fahlbusch, M. Rauh, Species differences of 11beta-hydroxysteroid dehydrogenase type 2 function in human and rat term placenta determined via LC-MS/MS, Placenta 37 (2016) 79–84.
[21] F.B. Fahlbusch, M. Ruebner, H. Huebner, G. Volkert, H. Bartunik, I. Winterfeld, A. Hartner, C. Menendez-Castro, S.C. Noegel, I. Marek, Trophoblast expression dynamics of the tumor suppressor gene gastrokine 2, Histochem. Cell Biol. 144 (3) (2015) 281–291.
[23] M. Ruebner, P.L. Strissel, M. Langbein, F. Fahlbusch, D.L. Wachter, F. Faschingbauer, M.W. Beckmann, R. Strick, Impaired cell fusion and differentiation in placentae from patients with intrauterine growth restriction correlate with reduced levels of HERV envelope genes, J. Mol. Med. (Berlin, Germany) 88 (11) (2010) 1143–1156.
[24] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis, Nat. Methods 9 (7) (2012) 671–675.
[25] L. Vićovac, J. Aplin, Epithelial-mesenchymal transition during trophoblast differentiation, Cells Tissues Organs 156 (3) (1996) 202–216.
[26] C. Hetheridge, A.N. Scott, R.K. Swain, J.W. Copeland, H.N. Higgs, R. Bicknell, H. Mellor, The formin FMNL3 is a cytoskeletal regulator of angiogenesis, J. Cell Sci. 125 (6) (2012) 1420–1428.
[27] T.E. Kreis, Microtubules containing detyrosinated tubulin are less dynamic, EMBO J. 6 (9) (1987) 2597.
[28] D.J. Kim, L.A. Martinez-Lemus, G.E. Davis, EB1, p150 Glued, and Clasp1 control endothelial tubulogenesis through microtubule assembly, acetylation, and apical polarization, Blood 121 (17) (2013) 3521–3530.
[29] G. Liao, G.G. Gundersen, Kinesin is a candidate for cross-bridging microtubules and intermediate filaments selective binding of Kinesin to detyrosinated tubulin and vimentin, J. Biol. Chem. 273 (16) (1998) 9797–9803.
[30] K.H. Yamada, Y. Nakajima, M. Geyer, K.K. Wary, M. Ushio-Fukai, Y. Komarova, A.B. Malik, KIF13B regulates angiogenesis through Golgi to plasma membrane trafficking of VEGFR2, J. Cell Sci. 127 (20) (2014) 4518–4530.
[31] R. Demir, Y. Seval, B. Huppertz, Vasculogenesis and angiogenesis in the early human placenta, Acta Histochem. 109 (4) (2007) 257–265.
[32] M. Langbein, R. Strick, P.L. Strissel, N. Vogt, H. Parsch, M.W. Beckmann, R.L. Schild, Impaired cytotrophoblast cell-cell fusion is associated with reduced Syncytin and increased apoptosis in patients with placental dysfunction, Mol. Reprod. Dev. 75 (1) (2008) 175–183.
[33] T. Todros, A. Sciarrone, E. Piccoli, C. Guiot, P. Kaufmann, J. Kingdom, Umbilical Doppler waveforms and placental villous angiogenesis in pregnancies complicated by fetal growth restriction, Obstet. Gynecol. 93 (4) (1999) 499–503.
[34] J.M. Roberts, C. Escudero, The placenta in preeclampsia, Pregnancy Hypertens. Int. J. Wom. Cardiovasc. Health 2 (2) (2012) 72–83.
[35] A. Wang, S. Rana, S.A. Karumanchi, Preeclampsia: the role of angiogenic factors in its pathogenesis, Physiology 24 (3) (2009) 147–158.
[36] Maynard SE and Karumanchi SA. Angiogenic Factors and Preeclampsia. p. 33–46.
[37] R.J. Levine, S.E. Maynard, C. Qian, K.-H. Lim, L.J. England, K.F. Yu, E.F. Schisterman, R. Thadhani, B.P. Sachs, F.H. Epstein, Circulating angiogenic factors and the risk of preeclampsia, N. Engl. J. Med. 350 (7) (2004) 672–683.
[38] A.S. Chung, N. Ferrara, Developmental and pathological angiogenesis, Annu. Rev. Cell Dev. Biol. 27 (2011) 563–584.
[39] J.H. Lim, S.Y. Kim, S.Y. Park, J.H. Yang, M.Y. Kim, H.M. Ryu, Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors, Obstet. Gynecol. 111 (6) (2008) 1403–1409.
[40] E. Soto, R. Romero, J.P. Kusanovic, G. Ogge, Y. Hussein, L. Yeo, S.S. Hassan, C.J. Kim, T. Chaiworapongsa, Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without placental lesions consistent with maternal underperfusion, J. Matern. Fetal Neonatal Med. 25 (5) (2012) 498–507.