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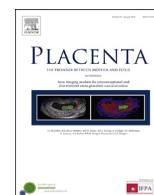
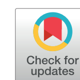


Detyrosinated tubulin is decreased in fetal vessels of preeclampsia placentas



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A B S T R A C T

Introduction: Preeclampsia is a hypertensive, gestational disease, which is still the leading cause of pregnancy related morbidity and mortality. The impairment of placental angiogenesis and vascularization is discussed to be of etiopathologic relevance. Deytrosination and tyrosination of α-tubulin is important for the stability and dynamics of microtubules. An increase of α-tubulin detyrosination leads to microtubule stabilization, which is an essential prerequisite for physiologic vascular tube morphogenesis during angiogenesis. So far, little is known about the specific localization of detyrosinated (detyr) and tyrosinated (tyr) tubulin in the placenta and its relevance for preeclampsia.
Methods: Placental expression of detyr- and tyr-tubulin was analyzed by immunohistochemistry, immunofluorescence and western blot. For western blot quantification we used biopsies from healthy placentas (n = 21) and placentas from pregnancies complicated with small for gestational age (n = 5), preeclampsia (n = 5) or both (n = 5).
Results: Specific placental localization of detyr-tubulin was detected in the fetal endothelial cells of the placenta. Villous and extravillous trophoblasts as well as villous stroma cells were tyr-tubulin positive. Detyr-tubulin protein expression was significantly decreased in placentas complicated by preeclampsia.
Conclusions: In summary, we report an accumulation of detyr-tubulin in villous vessels of the placenta and a significantly reduced level of detyr-tubulin in placental biopsies of preeclampsia cases. The reduction of placental detyr-tubulin in preeclampsia could suggest a deficit in villous vascular plasticity and might be associated with the impaired arborization of the disease.

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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

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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

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