Intussusceptive Growth of Vascular Bed in Human Placenta

İnsan Plasenta Damar Yatağının İntussusseptif Olarak Büyümesi

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ABSTRACT

Objective: Normal embryonic and fetal development is strictly bound to maternal health and functioning placenta. Besides the invasion and differentiation of trophoblastic cell lineage; development of effective vasculature is crucial for the function of placenta. Placental vessels first arise by vasculogenesis in early development of villi and then succeeded by angiogenesis during fetal life. In the recent decades a new form of angiogenesis, “intussusceptive angiogenesis”, besides classical sprouting angiogenesis is well documented. The presence of intussusception was shown at multiple organs but in placenta, in recent literature. We aimed to determine whether intussusceptive angiogenesis is present in human placenta to obtain further evidence on the development of vascular bed.

Methods: The term placenta samples were obtained from 10 healthy pregnancies following caesarean sections. Tissues were processed using routine plastic embedding technique; thin sections were contrasted with uranyl acetate & lead citrate; observed and photographed by transmission electron microscope.

Results: Our examinations revealed that both sprouting and intussusceptive angiogenesis is present in floating villi of term placenta. Phases of intussusception were documented in various samples.

Conclusion: The presence of intussusceptive angiogenesis will help our understanding of microvascular bed remodeling during pregnancy. We believe that this new finding will help us to determine the relation of microvascular bed development in normal and abnormal placentas.

Key Words: placenta, intussusceptive angiogenesis, sprouting angiogenesis, transmission electron microscopy

ÖZET

Amaç: Embriyo ve fetus gelişiminin normal olması anne sağlıklı ve plasentanın işlevi ile doğrudan ilişkilidir. Trofoblast hücre dizisinin farklılanması ve invazyonunun yanı sıra, etkin damar yatağının gelişmiş olması plasentanın fonksiyonu için kritik öneme sahiptir. Fetal hayat boyunca villusların erken geliş dönemi plasenta damarları ilk olarak vaskülogenezle oluşur ve daha sonra anjiogenesis ile devam eder. Sondakika “intussusseptif anjiogenesis” olarak tanımlanan yeni bir anjiogenesis modeli bildirilmiştir. Literatürde birçok organda intussusseptif anjiogenesis varlığı gösterilmiştir ancak plasentada böyle bir çalışma bulunmamaktadır. Çalışmamızda insan plasentasında intussusseptif anjiogenesisin var olup olmadığını belirleyerek damar yatağının gelişimine katkıları ile ilgili kanıt elde etmeyi hedefledik.

Hastalar ve Yöntem: On adet sağlıklı, term gebeden sezeryan doğumu takiben plasenta örnekleri toplandı. Doku örnekleri rutin plastik gömme yöntemi ile hazırlanarak, uranyl acetat ve lead citrate ile kontrastlandırılmış; geçirimli elektron mikroskop altında incelendi ve fotoğraflandı.

Bulgular: İncelemelerimiz sırasında term plasenta yüzey villuslarında anjiogenesisin hem tomurcuklanması ile hem de intussusseptif olarak gerçekleştiği gözlandığı, birçok örnekte intussusseptif anjiogenesisin çeşitli evreleri görüntüldiği belirlendi.

Sonuç: Intussusceptive anjiogenesis varlığı gebelik boyunca mikrovasküler yatağın yeniden mekanizmasına anlamlı bir yardımcıdır. İnanıyorum bu yeni gözlem normal ve anormal plasentalarda mikrovasküler yatağın gelişim iliskisini daha iyi anlamamızı yardımcı olacaktır.

Anahtar Sözcükler: Plasenta, intussusceptive anjiogenesis, tomurcuklama anjiogenesis, geçirimli elektron mikroskop

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INTRODUCTION

Placenta represents one of the highly vascularized organs to establish a critical efficient feto-maternal interface for the exchange of nutrients and waste products for normal development.

Formation of new blood vessels (neovascularization) is essential to achieve a functional microvascular bed that supports chorionic villi and extravillous trophoblastic cells and is necessary for the exchange of nutrients and gases for normal development of the growing fetus. However, the sources and mechanisms responsible for the angiogenesis that occurs during early stages of placental development are largely unknown (1). Sprouting angiogenesis is associated with vascular growth and remodeling during various physiological processes such as wound healing, tumor invasion, and angiogenesis in the developing heart (22), chicken chorio-allantoic membrane (23-28), human colon adenocarcinoma (29), in the peritoneal cavity and mesenteric tissue induced by tumor ascites fluid (30), ventricular muscle wall in developing heart in mouse embryos (31), in retina (27, 32), in mouse model of tissue repair (33), induced by tumor ascites fluid (30), ventricular muscle wall (21).

Sprouting angiogenesis (8-15) is a process in which endothelial cells extend cytoplasmic extensions of myofibroblasts, pericytes and finally interstitial fibers; and phase IV, growth of the slender pillar to a normal full size capillary mesh (21).

In addition to all angiogenesis types described above an additional mechanism for the development of new capillaries is also described as “intussusceptive angiogenesis”. This process of angiogenetic expansion of vascular bed, partition then elongation of existing capillaries takes place as reviewed by Djonov et al in detail (16). First findings on intussusception were reported by Short et al in 1950, by Ogawa et al in 1977 and Appell et al in 1980. In some capillaries sprouting angiogenetic endothelial cell clusters were observed in placental tissue samples that were obtained from healthy term placentas (age 20-40) who were followed in Department of Gynecology and Obstetrics, Baskent University Faculty of Medicine by cesarean section (C/S) with their informed consent approved by the Review Board and Ethical Committee of Baskent University (No: 14/56; dated 04/09/2014). The placental tissue samples were taken immediately after delivery and placed in 2% glutaraldehyde solution as first step. Samples were cut in to small pieces (1 mm3) and kept in 2% glutaraldehyde solution for 24 hours. Following primary fixation samples were washed with Sorenson phosphate buffer, and post-fixed in 1% osmium tetroxide for about an hour. Tissue samples were then dehydrated using graded ethanol series, substituted by propylene oxide and embedded in epoxy resin. Ultrathin sections (70-90 nm) were taken on copper grids using an ultramicrotome (Leica) and precipitated with uranyl acetate and lead citrate. The specimens were observed and photographed by LEO 906E (Zeiss, Germany) electron microscope.

RESULTS

We focused on the capillaries and related structures/cells in the floating villi of term placentas. All villi were rich in capillaries most of which exhibiting the fine structural features of mature capillaries with slender endothelial cells connected to each other by intercellular junctions, continuous basal lamina and associated pericytes.

Sprouting angiogenesis

In some capillaries sprouting angiogenetic endothelial cell clusters were present with a cleft-like narrow lumen. These ceboidal endothelial cells significantly rich in cytoplasmic filaments were connected to each other by tight junctions at their basilar compartments (Figure 1). In most cases we observed cross sections of these sprouts with similar structural features though it was not always possible to outline their original communication with parent capillaries due to section plane. These were frequently observed along the trophoblastic basal lamina at the peripheral compartments of floating villi. Their basal lamina was laminated in some cases, and some were associated with pericytes (Figure 2). More mature forms of these newly developing capillaries with a broader lumen were also observed. The thickness of endothelial cells in this vasculature gradually diminished surrounding a moderately developed lumen. However, these maturing endothelial cells were still relatively rich in cytoplasmic microfilaments. Lamination of the basal lamina surrounding these vessels and external lamina of pericytes were less prominent and lumina of some of them were occupied by red blood cells (Figure 3).
Intussusceptive angiogenesis

Besides sprouting angiogenetic figures described above we also observed structural features of intussusceptive angiogenesis in many examples representing phase I to IV of this remodeling process. In Phase-I samples, endothelial cells were observed to be extending towards the opposite wall some of which are already attached to each other by intercellular junctions forming pillars. In such newly established contact areas, endothelial cells were richer in cytoplasmic filaments like the endothelial cells of angiogenetic sprouts (Figure 4).

In other samples, processes of pericytes were projected between the attached endothelial cells crossing the original lumen of the parent capillaries representing a further stage of intussusception (Phase II-III). Endothelial cells crossing the lumen were similarly rich in cytoplasmic filaments reflecting a sign in their change in shape (Figure 5). We also determined migrating pericytes in intussusception areas surrounded by tiny intercellular matrix components resulting in relocation of intercellular junctions to allow this enlargement of the pillar area (Figure 6).
Figure 4: a) Electron micrograph of capillary within the villous stroma consisting of collagen fibers (Co) and cell processes. Upper right part of the capillary reflects the usual fine structural features mature patent capillary. However, the left side of the capillary has endothelial bridges (arrow) in the areas marked by dashed lines (Stage 1-2 of intussusceptive angiogenesis). Trophoblast basal lamina (*), interendothelial junctions (arrowhead), erythrocytes (e), Syncytiotrophoblast (Syn).
b) Higher magnification of stage 1. Interendothelial bridge (arrow). The cytoplasm of the endothelial cells at this special location is rich in cytoplasmic filaments (+). Intercellular junctions (arrow heads), erythrocytes (e).
c) Higher magnification of the marked area on the right (Stage II). Endothelial cells forming the bridge are rich in cytoplasmic filaments (+) similarly. Intercellular junction (arrow head) is also clearly distinguished. Collagen fibers (Co). (Uranyl acetate and lead citrate; original magnification a x2156, b x4646, c x10000)

Figure 5: a) Another capillary adjacent to vasculosyncytial membrane. Intussusception area marked by dashed line.
b) Higher magnification intercellular junction marked by arrow head and processes of pericytes (arrows) extend through the pillar. Syncytiotrophoblast (Syn), Cytotrophoblast (Cyt), Endothelial cells in the region are distinctly rich in intracytoplasmic filaments (+). (Uranyl acetate and lead citrate; original magnification a x2784, b x7750)
DISCUSSION

Growth and remodeling of placental vasculature is critical for normal fetal development. A newer form of angiogenesis, “intussusceptive angiogenesis” is recently described and attracted the attention of a number of researchers to this field in the last decades. We could not find a report about intussusception in human placenta. For this reason, we hypothesized that both sprouting and intussusceptive angiogenesis are efficient processes in human placenta and studied human placenta at electron microscopic level to test this hypothesis. We focused on the capillary sections in detail and obtained structural evidences of both angiogenetic mechanisms.

As efficient and sufficient maternofetal exchange is critical for normal development, many investigators studied the development and vascularization of placenta previously (2-6, 8-10). It is generally agreed that neovascularization starts with vasculogenesis which is followed by angiogenesis (2, 3, 7, 9). Several investigators introduced a number of terms like branching, non-branching sprouting etc. to define the formation of new blood vessels during further development (9-11). Intussusceptive angiogenesis attracted the attention of researchers thus several authors reported intussusceptive angiogenesis in a number of physiological conditions like branching, non-branching sprouting etc. to define the formation of new blood vessels method.

In the literature, changes of molecules (VEGF-A, PIGF, b-FGF, eNOS, O2, HIF, Tie1, Tie2, Ang-1, Ang-2) during vasculization in placenta is evaluated in both physiological and pathological pregnancies (5, 12, 14, 31, 42). But most of these studies are conducted in early placental development stages and they are focused on molecules rather than neovascularization types with the exception of a few studies. Mayhew et al., detected an increase in branching angiogenesis in pathologies like iron-deficiency anemia, high-altitude, hypoxia (11). Similarly, Soma et al., observed new vessel formation with angiogenesis during chronic hypoxia (43). Zhang et al., examined adaption, changes in diameter and perivascular cells of placenta vessels during pregnancy (14). Some authors identified intussusceptive angiogenesis and proposed that expansion of pillar is a mechanism that occurs for pruning useless/inefficient vessels (40, 44, 45).

In our study; we observed capillaries in all stages of intussusception in all of the samples examined. We focused on the floating (terminal) villi in which fetomaternal exchange of placenta is most represent the first report of intussusceptive angiogenesis in human term placenta. In conclusion; our findings reveal that further comparative studies in the placentas of maternal or fetal pathologies will be great value to understand the role of intussusception in the etiopathogenesis of a number of placental disfunctions.

Conflict of interest
No conflict of interest was declared by the authors.

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