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Tie2 expression in human embryonic tissues

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Abstract

Tie2 is a member of receptor tyrosine kinases family, involved in vasculogenesis and angiogenesis. Its main role is to stabilize, maintain, and facilitate the structural adaptation of the vasculature, during embryo development, and adult wound healing, or tumor development. Tissues from human embryos found in different stages of development (5 and 7-week-old), were investigated for Tie2 expression. The reaction was positive, with maximum intensity in the vascular cords, found in the mesenchymal tissue, and in the connective tissue around the primitive spinal cord. In the 7-week-old embryo, the reaction was negative in large blood vessels, and it was heterogeneous in those showing bridging phenomenon. In conclusion, during the first two months of human embryo development, we have concurrent vasculogenesis, angiogenesis, and blood vessel maturation and stabilization.

***Keywords*:** blood vessel stabilization, human embryo, neovascularization, Tie2.

Introduction

Endothelial receptor tyrosine kinases play an important role in the stabilization, maintenance, and structural adaptation of the vasculature [1]. Together with Tie1, Tie2 is restricted mainly to endothelial cells (ECs) and their progenitors [2]. During embryonic development, the expression of Tie2 is transcriptionally activated in EC precursors and maintained throughout embryonic endothelium. These receptors are essential to support the organization of the endothelial cells during embryogenesis, any disruption in Tie2 signaling resulting in embryonic lethality, secondary to defects in microvascular development [3, 4]. Although the expression of Tie2 is downregulated postnatally, it persists in quiescent mature ECs, in most normal blood vessels, including arteries, veins, and capillaries, suggesting a role for Tie2 in the maintenance and stability of quiescent adult vasculature [5]. Tie2 is upregulated in capillaries during neovascularization processes, including tissue repair and tumor growth, and downregulated, in regressing blood vessels [6, 7]. Most studies regarding embryonic vasculogenesis and angiogenesis were conducted on animal models. Because of the scarce information regarding the neovascularization in human embryo, we decided to investigate the expression of Tie2 in tissues obtained from embryos found in different stages of development.

Material and Methods

Five (n=3) and seven (n=2) weeks old human embryos were obtained after legal abortion, according to University's ethical guidelines. The gestational age was established according to Carnegie stages. After 48 hours fixation in 10% buffered formalin, and paraffin embedding, 5 μm cross sections, from the head and neck regions, were made. Each 10th slide was stained with Hematoxylin and Eosin, for morphological evaluation, and, subsequent slides, in chronological order, were immunostained with Tie2 antibody; the deparaffinized and rehydrated sections were heated in a microwave oven (in pH 6 citrate buffer) 20 minutes for epitope retrieval. Endogenous peroxidase was inhibited using 3% H_2O_2 for 5 minutes. The slides were incubated with mouse monoclonal Tie2 antibody (clone 9, Santa Cruz; 1:300 dilution). We used LSAB+ as working system, and the final reaction product was visualized, in a brown color reaction, with 3,3'-diaminobenzidine. All slides were immunostained automatically with the DAKO Autostainer. The nuclei were counterstained with modified Lillie's Hematoxylin, and the slides were mounted with a permanent medium (Canada balm). The results were assessed with a Nikon Eclipse 600 microscope. The images were captured and processed with Lucia G software system. We evaluated the distribution and intensity of positive reaction for Tie2 (+++, high; ++, medium; +, low; 0, absent) in different developmental stages embryonic tissues: mesenchymal tissue, primitive epidermis, blood vessels.

Results

On the successive embryo head and neck sections, we assessed the vascular structures with lumen, and vascular cords with lumen formation tendency.

On the Hematoxylin–Eosin stained preparations, the number, type and distribution of blood vessels depends on the developmental stage. In the 5-week-old embryo, the primordial dorsal aorta is already developed. Rare small blood vessels, vascular cords and vascular islands were found in the developing connective tissue around

the primitive nervous tissue, and peridermis. In the 7-week-old embryo, the vascular structures are more numerous in the differentiating connective tissue around the primordial spinal cord, and around future cartilage. In most of the cases is hard to differentiate between the mature and immature blood vessels.

In the 5-week-old embryo, Tie2 was positive, with maximum intensity and density, in the mesenchymal tissue, the reaction being less intense in the already differentiated connective tissue. We found no differences between mesenchyme and peridermis, regarding the expression and distribution of positive vascular structures: the vascular islands had peripheraly located Tie2-positive, and centraly located Tie2-negative cells. Immediately near the surface ectodermis the positive vascular structures were represented by vascular cords and blood vessels with lumen (Figure 1); some of the blood vessels were lined by Tie2-negative endothelial cells.

In some of the cases, isolated Tie2-positive cells were present aside small vessels lumens; we interpreted them as newly acquired pericytes. Compared to the mesenchyme beneath the surface ectodermis, where we found more blood vessels with lumen, in the mesenchyme located at the base of the neural tube, the Tie2-positive vascular cords were prevailing. In the same location we found spindle shaped, isolated, Tie2-positive cells. Immediately near the epithelium of primitive choroidal plexus, we found isolated Tie2-positive blood vessels, with isolated Tie2-positive endothelial cells on the contour. We also observed some Tie2-positive vascular cords in the embryonic tissues undergoing neural differentiation. The already developed primitive dorsal aorta was Tie2-negative. Around it, we found numerous isolated Tie2-positive cells and small blood vessels (Figure 2).

In the 7-week-old embryo, the intense positive reaction of the vascular islands and vascular cords in the mesenchyme is maintained. The endothelial cells lining the large blood vessels are Tie2-negative (Figure 3), with the exception of those lining blood vessels undergoing intussusceptive angiogenesis, where the reaction is heterogeneous; in some of these later cases, only the endothelial cells located in the vicinity of bridging zone are Tie2-positive, the rest being negative (Figure 4). Tie2 reaction is negative in the embryonic structures differentiating into nervous tissue, but is intensely positive in the vascular cords found in the committed mesenchymal tissue surrounding these structures. The differences in Tie2 expression between 5 and 7-week-old embryo are summarized in Table 1.

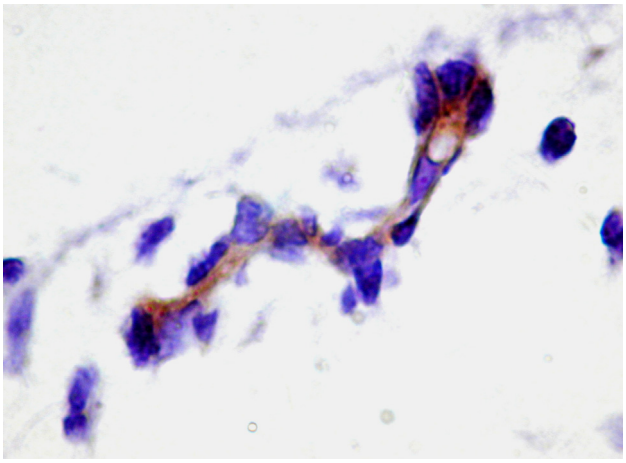

Figure 1 – ***Five weeks human embryo: intense positive reaction in the vascular cords, maintained also in vessels after lumen acquisitioning, ×400.***

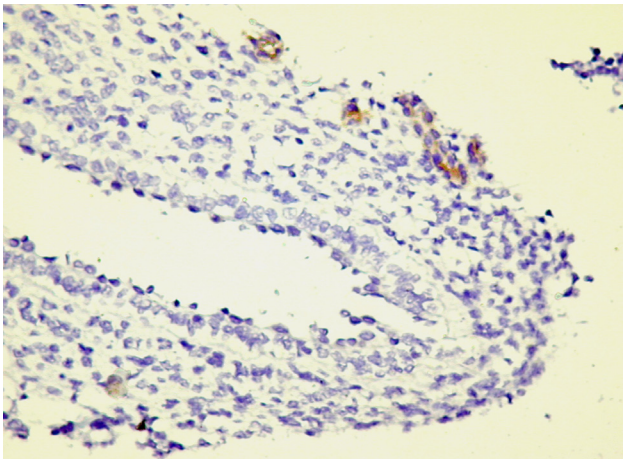

Figure 2 – ***Five weeks human embryo: negative reaction in the EC lining the dorsal aorta, with positive reaction in small vessels present in the wall, ×200.***

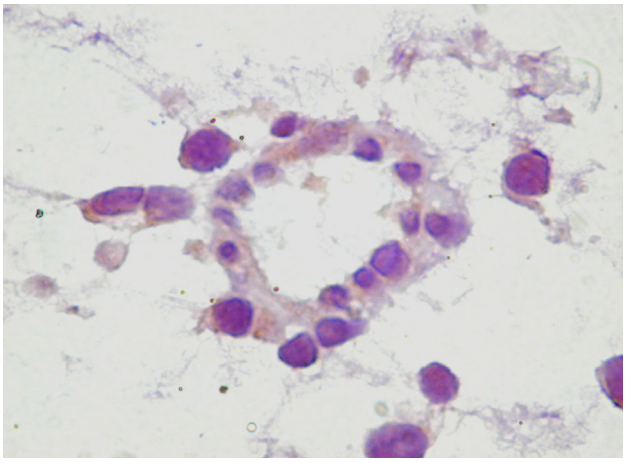

Figure 3 – ***Seven weeks old embryo: negative reaction in EC, with pale positive reaction in the mural cells of a mesenchymal large vessel, ×400.***

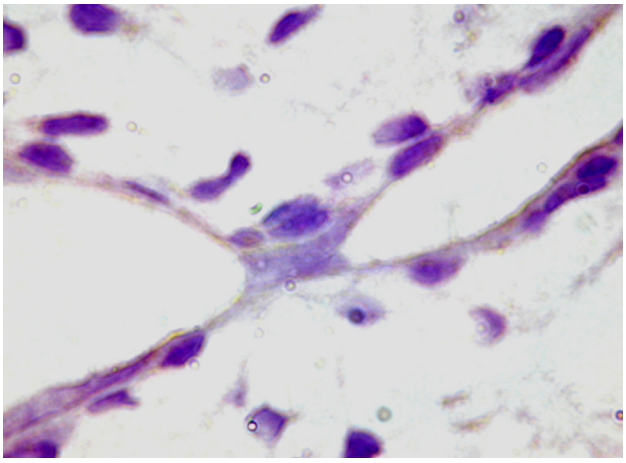

Figure 4 – ***Seven weeks old embryo: blood vessel with intussusception phenomenon-only the endothelial cells near the bridging zone are Tie2+, ×400.***

Table 1 – ***Intensity of Tie2 expression in embryonic mesenchymal tissues with different locations***

Tie2 expression / Mesenchymal tissue	5 weeks		7 weeks	
	Immature vessels	***Mature vessels***	***Immature vessels***	***Mature vessels***
Peridermis	+++	+/0	++	0/+
Around developing nervous tissue	++	0	+++	0
Developing nervous tissue	+	0	0	0

Intensity of immunohistochemical staining: 0, absent; +, low; ++, medium; +++, high.

Discussion

Tie2/angiopoietins system has been mainly evaluated in murine models [2, 3, 8]. Little data is available in the literature regarding Tie2 expression in human embryo. In our study, we evaluated the distribution and intensity of expression of Tie2 in the mesenchyme, and blood vessels developing in the differentiating mesenchyme of different gestational age embryos.

The blood vessel formation via vasculogenesis – differentiation of mesodermal cells to angioblasts, and subsequently to endothelial cells, or by angiogenesis – from preexisting blood vessels, during embryonic development or adulthood, requires several cell surface receptors as well as cell adhesion molecules [9, 10]. In both cases, formation of primitive blood vessel networks is completed by remodeling, and maturation of blood vessels through interaction between ECs and surrounding mural cells (pericytes or smooth muscle cells) [11]. Several of the most important receptors in both vasculogenesis and angiogenesis are tyrosine kinases. These receptors are prominently and selectively expressed in ECs. Recent studies [12], found Tie2 to be expressed not only by vascular structures, but also by tumor cells in some types of leukemia, gastric cancer, thyroid tumors, and inflammatory breast cancer. These findings open new possibilities in cancer treatment: the identification of inhibitors targeting kinases [13].

The Tie2 receptor and its ligands, the angiopoietins 1 and 2 (Ang1 and Ang2), are involved in blood vessel sprouting, remodeling, and integrity preserving. Ang1 is required for normal vascular assembly, by recruiting mural cells, and sustaining cell- cell contacts. Normally, Ang2 counteracts the action of Ang1 by competitively inhibiting its binding to Tie2 [4, 14]. It is not yet known the mechanism by which Tie2 transduces these different signals. There is thought to be a microenvironment-dependent receptor tyrosine kinase activation that may explain some of the different effects of angiopoietins in angiogenesis and vessel stabilization [14].

In our study, we found differences between the vascular structures found in the mesenchyme with different locations, and in the Tie2 expression in the blood vessels that acquired lumen. In the 5-week-old embryo, in the peripheral mesenchyme, we found vascular islands, cords, and blood vessels with lumen. Most of the vessels were Tie2-positive, but in the same location, we found also Tie2-negative blood vessels. In the mesenchyme around the developing nervous tissue, besides isolated Tie2-positive cells, we found mostly vascular cords, and some vessels that were Tie2-negative. These findings suggest the coexistence of vasculogenesis with resting, quiescent blood vessels.

In the more differentiated mesenchymal tissue of the 7-week-old embryo, the presence in the peripheral mesenchyme, along with vascular islands and vascular cords, of mature Tie2-negative vessels, with pale positive reaction in the mural cells, suggests the recruitment of pericytes [15]. In the same time, in the same location, we observed vessels undergoing intussusception that had a composite Tie2-positive reaction; these findings suggest the presence in the same time of stabilizing, maturing blood vessels, and destabilizing, undergoing angiogenesis blood vessels.

Conclusions

Our findings suggest that in 5 and 7-week-old embryos Tie2 has an intense, positive reaction in the vascular cords, a less intense positive, or even, a negative reaction in the mature vessels, and a composite reaction in the destabilizing, undergoing angiogenesis, vessels. In these early stages of embryonic development, vasculogenesis, angiogenesis, and vascular maturing are taking place in the same time, with zonal differences in vessel maturating stage, and Tie2 expression.

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