Review
Control of arterial branching morphogenesis in embryogenesis: go with the flow

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Abstract

Formation of a properly branched vascular system during embryogenesis is crucial for embryo survival. Here we review the regulation of the morphogenesis of the arterial and venous system during embryogenesis. We show that in addition to deterministic patterning mechanisms and plasticity of endothelial cells, arterial-venous differentiation and branching morphogenesis involves a prominent role for blood flow. Based on in vivo observations of developing arteries, we identified a novel morphological event crucial for the morphogenesis of the arterial tree, disconnection of small side branches. This disconnection of side branches occurs exactly at the point of bifurcation. The rate of disconnection of side branches depends on flow velocity and branching angle. The balance between disconnection and maintenance of arterial side branches determines the number of side branches connected to a large artery. Based on these observations, we postulate that the number of pre-existing collaterals connected to a large artery is a function of the disconnection process and can be regulated by hemodynamics. We furthermore show that embryonic arteries already adapt their lumen diameter to the amount of flow carried. Taken together, we suggest that hemodynamics plays a pivotal role in shaping the arterial system. We suggest that flow-evoked remodeling processes determine the number of preexisting collaterals during critical periods of embryo–fetal development. Insight into these basic principles of arterial growth and branching during embryogenesis may aid to understanding the observed variability in the capacity to establish a collateral circulation in patients with ischemic diseases and finding new strategies for therapeutic arteriogenesis.

Keywords: Developmental biology; Arterial–venous differentiation; Arteriogenesis; Endothelial receptors; Hemodynamics; Growth factors

1. Introduction

Understanding the orchestration of arterial–venous patterning and branching morphogenesis of the vascular system during embryogenesis is a great challenge. Several mechanisms regulating branching have been put forward, including the spatial distribution of attractive and repulsive guidance signals [1,2], nerves [3,4], genetic imprinting of arterial and venous identity in endothelial cells (EC) [5–7], and hemodynamics [8]. Especially, the observation that branched structures, like the nervous system and vascular system, share common ligands and receptors [4] has triggered a novel area of research aimed at elucidating control of branching. Understanding these basic principles of arteriolar growth and branching may aid in finding new strategies for therapeutic arteriogenesis. For example, arteriogenesis [9] involves outward remodeling of preexisting small arteriolar side branches and changes in the branching structure, resulting in a collateral circulation...
bypassing a stenosis. The question arises at what time point these preexisting side branches are formed during ontogenesis of the vascular system and how the amount of preexisting side branches is determined. We postulate that these aspects of arteriogenesis are determined during critical periods of embryo–fetal development and are highly regulated by hemodynamics. Here we address (1) the formation and remodeling of the embryonic vascular plexus from capillaries into branched arteries and veins and (2) hemodynamic vs. molecular control in the morphogenesis of branching, including a mechanism for regulation of the number of preexisting collaterals.

2. Formation of the embryonic vascular system

To understand the morphological processes and the molecular/hemodynamic control that results in de novo formation of arteries during embryogenesis, we briefly review (a) how endothelial cells differentiate and assemble into a primitive network, (b) the morphological processes needed to remodel the primitive plexus into branched arteries and veins, and (c) the involved control mechanisms. Vasculogenesis is the process that describes the in situ differentiation of endothelial precursor cells from the embryonic mesenchyme [10–12]. Vasculogenesis results in the formation of the earliest vascular plexus in the embryo, before the onset of perfusion. In the chick embryo, the first endothelial cells form during gastrulation and originate from lateral and posterior mesoderm [11]. Detailed analysis revealed that the posterior two thirds of the embryo, corresponding with the presumptive territories of lateral and posterior mesoderm, can give rise to endothelial cells and blood cells. Lateral and posterior mesodermal cells can migrate towards the yolk sac where they will differentiate to endothelial cells and to hematopoietic cells. During their migration, precursors aggregate to clusters termed hemangioblasts. Following differentiation of the yolk sac blood islands, endothelial cells surrounding these blood islands anastomose to form a capillary network. This network serves as a scaffold for the beginning of circulation.

Inside the embryo proper, two major vessels, the dorsal aorta and the cardinal vein, have differentiated. Embryonic circulation begins with the formation of the duct of Cuvier, which connects the cardinal vein to the extraembryonic circulation [13]. Blood pumped by the first heartbeats into the aorta flows back to the heart through the cardinal vein. However, in order to ensure nutrient supply to the embryo, blood must also flow through the yolk sac. This is achieved by the exit of arterial blood through the posterior end of the dorsal aorta into the yolk sac primary capillary plexus and backflow of venous blood through the peripheral yolk sac sinus vein [13]. As blood flow through the yolk sac increases, the yolk sac primary plexus is rapidly remodeled into arteries and veins in order to accommodate cardiac output.

At present, two morphologically distinct forms of angiogenic remodeling processes have been demonstrated: sprouting angiogenesis and intussusception [10]. Sprouting angiogenesis is the known classical form of angiogenesis and has been reviewed extensively [14,15]. Sprouting angiogenesis is controlled by growth factor gradients and specialized cells at the extremity of capillaries, the tip cells [2]. Tip cells contain growth factor receptors, most notably VEGF-R2 and extend filopodia that explore the local environment. Tip cells regulate capillary branching by following a gradient of extracellular matrix-bound growth factors that guide the endothelial cell invasion into and across the inhomogeneous tissue leading to the formation of lattice-like networks. The angiogenic growth factor VEGF is the major stimulator of this endothelial movement [2]. Heparin-binding forms of this growth factor have been shown to direct the tips of growing capillaries in several vascular beds, including capillaries invading the previously a-vascular hindbrain and retina during embryonic development [1,16]. Growth-factor-guided migration is of eminent importance for the vascularization of a-vascular regions and to lay down a primitive plexus from which arteries and veins can be generated. In zebrafish, it has been postulated that, during development, this mode of angiogenesis can (pre)determine positioning of major arteries, like aorta and intersomitic arteries [17–20]. The exact position of the intersomitic vessels as well as their growth velocity may depend on a delicate balance between attractive and repellant (guidance) cues. Tip cells display a remarkable functional similarity with growth cones of developing neurons. Since both the nervous and vascular systems are stereotyped as highly branched structures, the concept is emerging that branching of both systems could be controlled by common cues [4]. For the nervous system, it has been well established that axonal pathways are shaped by attractant and repulsive guidance cues. Studies in zebrafish demonstrated that the neuronal guidance receptor PlexinD1, which is normally involved in repulsive guidance in the nervous system, is also expressed in the vascular system and critically involved in the patterning of embryonic blood vessels [21] by guiding endothelial movement. It is tempting to speculate that differences in the ability to generate attractive and repulsive guidance cues contribute to the observed interspecies and interorgan variability in the number of preexisting collaterals.

In adult arteries, sprouting has not been reported, probably because mechanically, this is almost impossible, inasmuch as it would involve complete regression of several layers of smooth muscle cells in order for endothelial sprouts to be able to extend. It is more likely that, in the adult situation, terminal arterioles are formed when capillaries become invested with smooth muscle cells [22]. In this case, preexisting capillary pathways are “upgraded” into arterioles. Stimulating growth of capillaries can contribute to growth of small arterioles by
offering a larger pool of capillary segments to be potentially upgraded. The final number of arterioles, however, is still being dependent on the upgrading process or the recruitment of vascular smooth muscle cells in the vessel wall. Next to VEGF, other growth factors, especially from the FGF family, have been implied in arteriolar network development. In the developing chick embryo heart, both VEGF and FGF-2 have been shown to stimulate myocardial vasculogenesis [23]. Additional studies using neutralizing antibodies against VEGF and FGF-2 showed that both growth factors are involved in capillary growth, but only FGF-2 appeared to facilitate growth of arterioles [24]. This probably relates to the potent effect of FGF-2 on vascular smooth muscle cell proliferation and migration (critical factors in the capillary potent effect of FGF-2 on vascular smooth muscle cell growth of arterioles [24]. This probably relates to the capillary growth, but only FGF-2 appeared to facilitate growth of arterioles [24]. This probably relates to the capillary growth, but only FGF-2 appeared to facilitate growth of arterioles [24]. This probably relates to the capillary growth, but only FGF-2 appeared to facilitate growth of arterioles [24]. This probably relates to the capillary growth, but only FGF-2 appeared to facilitate growth of arterioles [24]. This probably relates to the capillary growth, but only FGF-2 appeared to facilitate growth of arterioles [24]. This probably relates to the capillary growth, but only FGF-2 appeared to facilitate growth of arterioles [24]. This probably relates to the capillary growth, but only FGF-2 appeared to facilitate growth of arterioles [24]. This probably relates to the capillary growth, but only FGF-2 appeared to facilitate growth of arterioles [24].

Intussusceptive angiogenesis or nonsprouting angiogenesis relates to a process that splits (like a “zipper”) existing vessel segments by transcapillary pillars, resulting in the division of the vessel and generation of two separate segments with comparable diameter [27,28]. Intussusception can be regulated by hemodynamics [28,29], but the molecular control mechanisms remain unclear. In the developing chick embryo yolk sac, intussusceptive arborisation and intussusceptive branching remodeling are abundant, while angiogenic sprouts are present in the primary capillary plexus but only rarely observed during the arterial–venous remodeling phase [8]. At present, it is not understood how a vessel determines its growth mode, sprouting angiogenesis, or intussusception.

3. Arterial–venous differentiation

Based on classic studies, it was believed that endothelial cells formed by vasculogenesis constitute a rather homogenous group of cells. Over the last few years, however, several signaling molecules were discovered, which differentially label arterial or venous endothelial cells from early developmental stages onward, prior to the onset of circulation. Interestingly, many of these molecules are also expressed in the nervous system where Notch is implicated in cell fate decisions, the Notch pathway in endothelial cells thus appears to specify arterial fate.

4. Plasticity of endothelial cells with respect to arterial–venous differentiation

In spite of the implication of the genes described above in establishing arterial or venous fate, several recent studies suggest that endothelial cells are not genetically committed to an arterial or venous phenotype but are plastic and adapt to the expression of the arterial or venous specific genes based on local environmental cues [37,48]. This was first suggested by transplantation studies where fragments of arteries and veins were isolated from quail hosts and implanted into chick embryos. The fate of the grafted cells could be traced using a monoclonal antibody recognizing all quail endothelial cells. It was found that arterial endothelial cells could colonize veins and vice versa. Importantly, expression of arterial and venous-specific genes changed according to the novel environment.

In a recent study, we used a time-lapse video microscopy system and examined arterial–venous differentiation in the developing yolk sac of chick embryos [8]. The chick embryo is a standard model in embryogenesis and allows easy access to the vasculature and pharmacological or mechanical manipulation. We observed that prior to the...
onset of flow, endothelial cells expressing arterial or venous-specific markers are localized in a posterior–arterial and anterior–venous pole [8]. After the onset of heartbeat and perfusion, the vitelline artery forms in the posterior arterial pole. Formation of the vitelline artery from the arterial capillary plexus occurs by flow-driven fusion of capillaries branching off the aorta at the level of around somite 21. Capillaries branching from the aorta at levels posterior to somite 21 concomitantly receive less flow and are selectively disconnected from the aorta. These disconnected segments rapidly lose expression of arterial markers and are subsequently used to form the vitelline veins [8]. Thus, during normal development, previous arterial vessel segments are used to form veins. Interestingly, during this period of arterial–venous differentiation, only very little apoptosis was observed in blood vessels, suggesting that endothelial cells can be used and reused to fashion growth of arteries and veins in the rapidly expanding yolk sac vasculature. It thus differs from the classical pruning concept since the cells do not die and are not removed from the vasculature. In zebrafish, the development of intersomitic arteries and veins also appears to involve disconnection and reconnection of vessel segments [49], suggesting that this mechanism may be used in different species and developmental settings.

These observations suggest (1) a requirement for endothelial plasticity with respect to arterial–venous differentiation for normal vascular development and (2) a crucial role for hemodynamics in the process of arterial–venous patterning. To test these hypotheses, we performed several flow manipulations [8]. Most strikingly, ligation of one vitelline artery by means of a metal clip, lifting the artery, and arresting arterial flow distal to the ligation site could morphologically transform this artery into a vein. Expression of mRNAs for the arterial markers ephrin-B2 and neuropilin-1 was found to be rapidly down-regulated following the ligation, whereas venous markers, including neuropilin-2, were up-regulated. Restoration of arterial flow by removal of the metal clip could restore arterial marker expression, suggesting that the genetic make-up of arterial endothelial cells is plastic and controlled by hemodynamics [8].

An important clinical implication of these observations is the ‘arterialization’ of veins following coronary bypass grafts.

5. Disconnection of side branches: a key mechanism to generate preexisting collaterals?

A crucial feature of vitelline artery formation in the yolk sac is the disconnection of small side branches. This process allows the transformation of the capillary plexus into larger and smaller tubes. Flow is thus distalized throughout the yolk sac, which is essential for nutrient uptake and oxygenation during expansion of the embryo.

To understand this process in more detail, we performed in vivo studies using high-magnification intravital microscopy [8]. We observed that, exactly at the point of the bifurcation, the lumen of the side branch is progressively narrowed, leading to the complete closure of the side branch (Fig. 1). At this point, the side branch is now disconnected from the main branch and does not carry flow but remains pressurized through connections with more distal parts of the capillary plexus (Fig. 1). The disconnected segments do not regress but start to form sprouts that grow across the artery towards small veins of the primary circulation [8] (Fig. 1). Once the sprout fuses with the primary veins, the segment is perfused again through its connection with the distal part of the arterial tree (Fig. 1). Thus, vessels initially part of the arterial domain are disconnected and incorporated in the venous network; they are essential to fashion growth of embryonic veins. In the absence of flow, branched arterial–venous networks do not develop in the yolk sac, suggesting that flow is necessary to drive arterial–venous differentiation in this tissue during this stage of development [8].

Taken together, in the chick embryo yolk sac, arterial branching remodeling is not genetically determined but the result of a self-organization process regulated by flow. Disconnection of side branches plays a pivotal role in establishing a properly branched arterial tree. It results in the formation of capillary free zones around arteries, which can

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Fig. 1. Schematic representation of the disconnection–reconnection process. Schematic representation of the initial vessel configuration (top left) in the chick embryo yolk sac plexus; note that the main branch has numerous side branches. Arrows indicate flow direction; arterial domain, red arrows; venous domain, blue arrows. During subsequent stages of development (top right), side branches are selectively disconnected. In the disconnected segments, stasis spots appear. Subsequently (bottom left) from these stasis spots, lumenized and pressurized vessel sprouts project towards the primary venous plexus. Finally (bottom right), sprouts reconnect with the veins of the primary plexus. Previously disconnected segments are still connected to the arterial domain through distal connections. Once the connection with the venous system is established, flow is reintroduced in the disconnected segments, which now become part of the venous system. For colour indication we refer to the on-line version of this article.
readily be observed in a variety of vascular beds, including the mouse retina [2,50], suggesting that it is a common phenomenon. However, some branches are not disconnected but remain [8]. The number of arterial side branches branching from the main artery is a function of the efficacy of the disconnection process. To understand the conditions that can lead to persistence of side branches, we used a physical modeling approach integrating in vivo observations in a simple elastoplastic deformation model using a finite element method [51–55]. The physical modeling data show that disconnection of side branches depends on the flow velocity difference between the main artery and the side branch, and the branching angle (Fig. 2). At the branch point proper, flow induces elastoplastic deformations of the vessel wall that tend to close side branches in arterial diverging bifurcations. In the arterial tree, side branches are likely to be disconnected when the flow velocity difference between main and side branch is large, or the branching angle is blunt (above 90°). These morphological events, evoked by flow or shear, are of physical origin and depend on the mechanical equilibrium between the vessels and the surrounding interstitium. Taken together, these studies clearly show that the balance between disconnection and maintenance of arterial side branches determines the number of side branches connected to a large artery. We postulate that the number of preexisting collaterals in the context of arteriogenesis after arterial occlusion depends on this flow-regulated process. It may thus be a direct function of the fetal hemodynamic conditions and be influenced by adverse intrauterine conditions [56]. Thus, the genetic control of the number of preexisting arterial side branches could be exerted indirectly through molecular regulation of fetal hemodynamics and fetal volume handling.

In general, the disconnection is necessary since persistence of the initial vascular configuration in the yolk sac would preclude progression of blood flow into more distal areas, resulting in proximal arterial–venous shunting of blood, and would hence be detrimental for embryonic development. To ensure nutrient and oxygen uptake, blood flow to the capillary plexus (i.e., the exchange area) has to be ensured. This distalization of flow is achieved by disconnection of small arterial side branches from the larger arteries as they grow. During development, the process repeats itself in a proximal to distal progression permitting outgrowth of the arterial tree.

6. Flow and vessel caliber

Next to the regulation of the principal branching structure, blood flow also plays a pivotal role in the local regulation of vessel lumen of individual vessel segments. Already 100 years ago, Thoma [57] noted that vessels carrying a lot of flow widen, whereas vessels with low flow regress. At present, it is well established that adult vessels acutely adapt their vessel lumen to shear stress [58–60]; that is, higher shear stress increases arterial lumen diameter and vice versa. In this way, mean shear stress is kept within limits. In fact, shear stress is considered to be the driving force behind arteriogenesis [9]. However, it is not known whether embryonic arteries also adapt their vessel caliber in order to maintain constant shear levels. To address this question, we designed a video-microscopy setup that allows the measurement of red blood cell velocity in vivo [8,61]. By means of this in vivo intravital video-microscopy approach, we were able to measure red blood cell velocity [61] and arterial lumen diameter in the intact developing vitelline arterial circulation of the chick embryo during the critical period of arterial–venous differentiation. In absolute values, red blood cell velocities ranged from about 2–3 mm/s at the transition of the dorsal aorta to the proximal part of the vitelline artery to as low as 50 μm/s in the distal part. In the proximal parts of the arterial tree, the red blood cell velocity pattern displayed both positive (during systole) and negative (during diastole) velocities, indicating that, during one heart cycle, there is both

![Fig. 2. Shear evoked adaptation at the vessel bifurcation. Schematic representation of a vessel bifurcation (top left). Classical models are based on adaptation of vessel diameter to flow. The flow in each tube is supposed to be Poiseuille like. In current models, the variation of diameter is controlled by shear and pressure on the lumen side of the tube [77]. The adaptation of the vasculature amounts to solving Kirchhoff’s law with varying resistances of the strands. Thus, as flow in one vessel varies, so does its diameter over the entire length between the vertices (top right, arrow). In contrast, we observed (middle) that side branches are disconnected from the main branch while the diameter of the side branch has not significantly changed [8]. Exactly at the point of bifurcation, a selective narrowing of the vessel lumen can be observed (middle, arrow). The narrowing of the vessel lumen at the bifurcation can be explained using an elastoplastic adaptation model (bottom). Closing of the side branch will preferentially occur in a narrow region around the interconnection where the assumption of Poiseuille’s law breaks down. The closing velocity is dependent on the flow velocity difference between main and side branch, and the branching angle (bottom). Dotted line represents a branching angle of 65°; straight line represents a branching angle of 90°.](https://academic.oup.com/cardiovascres/article-abstract/65/3/619/355193)
forward and backward movement of flow in the arteries (Fig. 3). This particular velocity pattern may be the result of the functional anatomy of the heart, because at this stage, heart valves have not fully developed yet. Since the time period and the velocity of antegrade flow exceed the period and the velocity of retrograde flow, there is still a net forward movement of blood averaged over the cardiac cycle. In general, in the distal parts of the arterial tree, periods with backward flow were absent, and forward flow prevailed. The velocity pattern, however, is still pulsatile because of flow arrest (red blood cell velocity of zero) at the end of diastole. From velocity and vessel diameter, as assessed in vivo, reduced velocity ($U$) was calculated. Reduced velocity is a first-order approximation of actual shear rate, i.e., $dV/dr$, where $V$ is velocity and $r$ is vessel radius. If embryonic arteries adapt their lumen size to the amount of flow carried, for a given vessel diameter, shear stress levels should be constant. We indeed observed that, in embryonic arteries, reduced velocity was constant, with $U$ averaging about 10 s$^{-1}$, within a diameter range of 40–200 μm (Fig. 3). These in vivo data show that embryonic arteries already adapt their lumen diameter in response to shear stress. Interestingly, at this stage of development, a vessel wall media, differentiated vascular smooth muscle cells, and active regulation of vascular tone are still lacking. Thus, even in the absence of vascular smooth muscle, arteries only consisting of endothelial cells adapt their structure to shear rate and, hence, shear stress. This implies that the organization of developing tube structures is already optimized to the amount of flow to be carried [62] and is controlled by the endothelium. The disconnection process plays a pivotal role because it blocks the “leakage” of volume to the side branches, thus allowing the volume to remain in the arterial tree and hence allowing shear induced outward remodeling of the arteries. Without the disconnection process, the arterial tree is like a rubber tire with lots of holes; filling does not inflate the tire unless you close the holes. Specific differences in shear stress levels or velocity patterns between arteries and veins may underlie the induction or maintenance of differential expression of arterial- and venous-specific genes in the endothelium of these domains. In vitro experiments with endothelial cells exposed to different shear stress stimuli followed by gene array analysis indeed suggest that this may be the case [63–65]. To summarize, we conclude that, during early embryonic development, arteries adapt their lumen size to the amount of flow carried and maintaining mean shear stress at a constant level. During embryonic development, flow is capable to influence both arterial segment caliber and arterial topology.

7. Integrating view on deterministic and environmental cues in shaping the vascular tree

Based on experimental data and on conceptual considerations, it can be assumed that generation of organ-specific vascular structures involves a combination of processes (a) based on genetic predetermination and (b) involving a feedback by parameters linked to the function of the generated vascular structures within the organ. In the context of this review, genetic predetermination would mean that vessels are generated, which at the time of their generation are not necessary to maintain tissue function and which may not be perfused. In contrast, functional feedback control implies a change in vascular structure in response to hemodynamic stimuli provided by the flowing blood and metabolic stimuli reflecting the supply/demand relation of the tissue supplied. The characteristics of both mechanisms correspond to specific requirements during different stages of development and different types of structures generated.
In embryonic development, the basic layout of the cardiovascular system is made very early and at a stage where its components are not functional according to their destined purpose. As circulation can only be of functional relevance after the establishment of a pumping heart, arterial trees, capillaries (or arterio–venous flow pathways), and veins, the initial generation of these components cannot be guided by their target functions. Similar arguments hold for the generation of other organ systems, e.g., the neural system. Here, only a predetermined pattern generation mechanism can work. A striking example is the generation of the aorta and cardinal vein and its primary branches in zebrafish [66]. However, this predetermined growth is ‘blind’ with respect to its actual functional consequences, and small changes in the local environment or genetic program may lead to catastrophic deviations from the original ‘blueprint’ [21]. Thus, it makes a lot of biological sense to use parameters derived from the physiological function of the organ system to fine-tune and control further development as soon as they become available after the basic structural layout has been achieved. For the vascular system, these parameters would include blood flow and blood pressure, and the related physical forces wall shear stress and circumferential wall stress as well as parameters reflecting the metabolic state of the tissue (e.g., PO2).

Thus, the balance between predetermined and functionally coupled control of vascular structure is influenced by developmental stage and hierarchy of the structures considered. While the early generation of primary structures is predominantly predetermined and these structures show little interindividual variation, structural adaptation at later stages, up to and including the adult, of more downstream components relies mainly on functional feedback. As a result, these structures exhibit a large heterogeneity both within and between individuals, but they are fit to serve the functional needs.

8. Architecture of arteriolar trees

For arterial trees, two distinct branching patterns can be recognized, the dichotomous tree and the anastomosing or arcading tree (Fig. 4). In the dichotomous tree, the main branch gives rise to two daughter branches that subsequently branch in a similar way until the capillary level is reached. The dichotomous branching pattern may show fractal characteristics. Typical examples are the coronary tree and the arterial system in the chick embryo chorioallantoic membrane (CAM). In the anastomosing or arcading network, one branch point may connect to more than two side branches that subsequently reconnect with other segments of the same tree, resulting in a polygonal network appearance. Arcading networks can readily be observed in the intestinal (mesenteric) circulation, skeletal muscle, and the thermoregulatory part of the skin vasculature. Arcading structures are often seen in organs frequently compressed or distended when alternative flow pathways are helpful to maintain tissue nutritive flow and to regulate temperature.

With respect to arterial occlusion, the advantage of an arcading arteriolar network is obvious. Stenosis anywhere in the tree does not result in compromised flow in distal areas since collateral channels that can take over flow are readily available. The molecular mechanism accounting for tissue-specific naturally occurring arcade formation in vascular beds, like skin and intestine, is as yet unclear. For the skin, recent experimental data in mice suggest that, during embryonic stages E12–E16, sensory nerves may play a pivotal role in prepatterning arterial network morphogenesis [3]. It was shown that sensory-nerve-derived VEGF induces capillary expression of the arterial marker ephrin-B2 and subsequent remodeling into small arteries. It was concluded that the spatial distribution of the sensory nerves determines the arteriolar remodeling process. However, nerve-guided arteriolar patterning is not generalized; during embryogenesis, most vascular beds, including the yolk sac, are established before the arrival of nerves, while other vascular beds (like the CAM) never see any nervous innervation [13]. It is currently unknown if distinct morphogenetic mechanisms (nerves, growth factor guidance, disconnection–reconnection) lead to the formation of dichotomous or arcading branching patterns.

Fig. 4. Branching patterns in the chick embryo chorioallantoic membrane (CAM). Top: Normal arterial branching pattern observed in the chick embryo CAM at day 14 of development. Note the dichotomous branching structure; only very few anastomosing vessels are observed. Bottom: Arcading branching pattern as observed in the CAM at day 14 of development after 7 days of treatment with angiotensin-II (modified after Ref. [71]). Note the emergence of numerous arcading vessels.
arcading trees. The challenge for future research is to obtain an integrative view on the interaction between the morphogenetic events, including tip cell guidance, intussusception, disconnection–reconnection, the molecular key players, and, once perfusion is established, the emergence and maintenance of a stable network configuration.

For a dichotomous tree, arterial occlusion results in principle in a severe reduction of flow delivery to the distal parts, the magnitude depending on the site of occlusion. In this case, flow can only be maintained in the presence of preexisting collateral arteries, as may be the case in the coronary circulation. Theoretically, in the absence of preexisting collaterals, restoration of flow can also occur if adjacent arteriolar trees, proximal and distal to the stenosis, form an arcing or collateral connection. In general, such a process will require more time than opening of preexisting collaterals. It has been postulated that, under normal flow conditions, these types of arcing arterioles are formed when terminal arterioles from adjacent transverse arteriolar trees cross along back-connecting capillaries and connect to each other [67,68]. Thus, existing capillary vessels interconnecting adjacent arteriolar trees are upgraded to arterioles [22]. This process of de novo arteriolization can be the result of direct effects of growth factors (see Fig. 4) or of local hemodynamic changes [69].

We previously demonstrated that application of the growth factor angiotensin-II transforms the dichotomous arteriolar branching pattern of the chick embryo CAM into an arcing network [70,71] (Fig. 4). In this setting, arcade formation is caused by arteriolization (adding layers of smooth muscle cells) of previously existing capillary segments interconnecting adjacent arterial trees. The clinical relevance of such observations became clear in a recent study, in which angiotensin-II was shown to stimulate collateral vessel growth in peripheral ischemic disease [72]. At the molecular level, angiotensin-II exerts a direct growth effect on vascular smooth muscle cells while indirectly affecting the stability of endothelial cells by stimulating the release of VEGF [73]. The hemodynamic mechanisms underlying arcade formation have been addressed in a series of mathematical modeling studies [74]. From these theoretical considerations, it was concluded that, under normal flow conditions, pressure-associated circumferential wall stress adaptation can induce upgrading of capillaries into arterioles resulting in an arcade vessel between two adjacent arteriolar trees [75]. This concept on the role of pressure in the adaptation of arcade networks has recently been shown to hold in vivo [68]. In the case of arterial occlusion, i.e., in the setting of ischemia, however, these assumptions may not hold, and instead, adaptation to shear stress may become the dominant control mechanism [9]. Flow-induced outward remodeling of arteries (lumen enlargement of existing arteries) and upgrading of capillaries into small arterioles have been well established [9,76], and it is clear that shear stress can activate the necessary molecular pathways and gene expression essential to the remodeling process [63,65]. To summarize, factors suggested to contribute to the formation of arcing vessel networks include pressure and wall stress adaptation, growth factors like angiotensin-II, and, possibly, sensory nerves.

9. Conclusions

Generation and maintenance of a functionally adequate arterial system involves two different types of mechanisms. The basic layout of major vessels in very early phases of development, prior to the onset of perfusion, may be regulated by deterministic patterning mechanisms. Upon establishment of flow and exchange with the developing tissue, respective signals are vital for the evolution of structure and function of the vascular system. Flow and flow-related shear stresses, together with hemodynamic signals such as pressure, are specifically involved in controlling vessel caliber and the number and position of arterial side branches and thus in the topology of the generated arterial trees and the number of preexisting collaterals. Regulatory effects of flow on vascular structure and function thus have to be taken into account in any analysis of vascular development. Improved understanding of the shear dependent mechanisms and their interaction with predetermined patterning, reactions to angiogenic factors, local metabolic stimuli, and hemodynamics could provide a basis for future therapeutic strategies addressing vascular malfunction.

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