Three-Dimensional Analysis of Vascular Development in the Mouse Embryo

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Abstract

Key vasculogenic (de-novo vessel forming) and angiogenic (vessel remodelling) events occur in the mouse embryo between embryonic days (E) 8.0 and 10.0 of gestation, during which time the vasculature develops from a simple circulatory loop into a complex, fine structured, three-dimensional organ. Interpretation of vascular phenotypes exhibited by signalling pathway mutants has historically been hindered by an inability to comprehensively image the normal sequence of events that shape the basic architecture of the early mouse vascular system. We have employed Optical Projection Tomography (OPT) using frequency distance relationship (FDR)-based deconvolution to image embryos immunostained with the endothelial specific marker PECAM-1 to create a high resolution, three-dimensional atlas of mouse vascular development between E8.0 and E10.0 (5 to 30 somites). Analysis of the atlas has provided significant new information regarding normal development of intersomitic vessels, the perineural vascular plexus, the cephalic plexus and vessels connecting the embryonic and extraembryonic circulation. We describe examples of vascular remodelling that provide new insight into the mechanisms of sprouting angiogenesis, vascular guidance cues and artery/vein identity that directly relate to phenotypes observed in mouse mutants affecting vascular development between E8.0 and E10.0. This atlas is freely available at http://www.mouseimaging.ca/research/mouse_atlas.html and will serve as a platform to provide insight into normal and abnormal vascular development.

Introduction

The cardiovascular system is the first functional organ system to develop in the mammalian embryo. The blood vessels that initially comprise this organ originate by vasculogenesis, the aggregation of de novo-forming angioblasts (endothelial precursors) into simple endothelial tubes. Angioblasts in the mouse embryo first emerge from the mesoderm as Flk1+ cells around embryonic day (E) 7.0 and assemble a simple circulatory loop consisting of a heart, dorsal aorta, yolk sac plexus and sinus venosus by E8.0 [1,2,3]. Shortly after its formation, this early vascular circuit is remodelled by angiogenesis, the proliferation, sprouting and pruning of pre-existing vessels, transforming it into a complex network of branched endothelial tubes of varying diameter, length and identity. Such remodelling of pre-existing vessels is dependent on both genetically hardwired events and hemodynamic forces [4,5].

Given the complex nature of the vascular system and the diversity of biological processes required for its assembly and refinement, it is hardly surprising that a large number of signalling pathways are employed in its development. Mutations in pathways required for vascular development frequently manifest phenotypes that result in embryonic lethality at mid gestation. In mice, mutations affecting Notch [6,7,8], TGFβ [9,10], Hedgehog [11,12,13], VEGF [14,15,16], ephrin/Eph [17] and angiopoietin/Tie [18] signalling (among others) result in abnormal vascular development between E8.0 and E10.0 and ultimately embryonic lethality. The vascular activities of these pathways are not limited to this developmental time window, but extend to organogenesis [19,20], maintenance of vascular homeostasis in adulthood [8,9,21,22] and states of pathological angiogenesis [23,24,25,26]. Correct interpretation of how these pathways regulate vascular development between E8.0 and E10.0 would therefore improve our understanding of how they contribute to later vascularization events. Such interpretation is often impeded however, by the complex nature of the vascular phenotypes, an inability to observe the vasculature of the mutants in its entirety and an incomplete understanding of the normal sequence of vascular remodelling events that occur during this period of development. Previous studies in zebrafish [27,28] and chick [29,30] have provided insight into normal vascular development, but have limited applicability to the sequence of vascular remodelling events in the mammalian embryo primarily due to differences in anatomy.
and the increased use of plexus bed intermediates in mammals compared to zebrafish. We have sought to address this issue by generating a high resolution, three-dimensional (3D) atlas of the developing mouse vasculature between E8.0 and E10.0 (5–30 somites).

The mouse embryo grows rapidly between E8.0 and E10.0 and undergoes complex morphological and conformational changes that present significant challenges to current imaging technologies. These challenges are further complicated by the inherent properties of the vascular system as a 3D network of branched, interconnected tubes of varying length and size. Accurate assessment of vascular development at this stage therefore requires a 3D imaging modality capable of visualizing the vasculature in its un-manipulated entirety in embryos of increasing size while retaining sufficient isotropic resolution (on the order of a few microns) to capture the details of the finest capillaries. Without these properties, significant positional information about the vasculature is lost and artefacts are introduced. While confocal microscopy has been used to generate an atlas of vascular development in zebrafish embryos [27] and study projections of the vasculature of dissected mouse embryos prior to E8.5 [2], it does not provide sufficient specimen coverage to create the 3D images necessary to visualize the complete embryonic vasculature in mouse embryos beyond this time point. Later embryonic stages have been studied using corrosion casting and electron microscopy [31]. While these investigations provided 3D representation, they did not retain information about vessels that cannot be perfused such as blind ended angiogenic sprouts, narrow vessels and vessels yet to form a complete lumen.

Recently, a new imaging modality named Optical Projection Tomography (OPT) [32] was developed to obtain molecular specificity and 3D cellular level resolution over complete specimens up to 1 cubic centimetre (cc) in size. OPT has been shown to support the use of multiple molecular markers [32], and has been previously used to visualize mouse embryos [32,33], chick embryos [34], developing plant material [35], drosophila melanogaster [36] and whole adult mouse organs [37,38]. We have further developed OPT using frequency-distance relationship [39] (FDR)-based deconvolution to obtain higher resolution images, on the order of a few microns, while still retaining the ability to image large specimen sizes [40,41]. We set out to use this technique to study the normal sequence of mouse vascular development between E8.0 and E10.0.

Ideally one would be able to image a single living mouse embryo over an extended period of time in order to visualize the complete development of a single vasculature. 3D optical imaging of the live mouse embryo, however, is still a challenge due to the in vivo development of mouse embryos and significant light scattering caused by living tissue. This in vivo time course can instead be approximated by static imaging of multiple genetically identical embryos collected at a range of ages throughout gestation. To this end, we performed FDR-based deconvolution OPT imaging on fixed PECAM-1-immunostained embryos ranging from 5 to 30 somites to study the normal development of the early embryonic mouse vasculature. PECAM-1 is major constituent of the endothelial cell intercellular junction and is widely used as a molecular marker for mature endothelial cells [2,42,43,44]. With these images we are able to visualize vascular development across this age range, from vasculogenesis to capillary plexus to the development of larger vessels. The collection of all images comprises an atlas of normal development of the embryonic mouse vasculature, which is made freely available to other researchers. Analysis of this atlas has resulted in significant new information regarding the normal vascular development in the mouse embryo.

### Results and Discussion

Acronyms for the vessels discussed in this paper are listed in Table 1.

#### 3D Visualization of the Embryonic Mouse Vasculature

A sample OPT view of the Cy3-PECAM-1 signal from a 19 somite embryo is shown in Figure 1. At 0.9 degree angular increments of the specimen, 400 views were taken throughout a complete revolution. These views were then FDR-filtered as described (see Materials and Methods) and reconstructed using a standard parallel-ray filtered backprojection reconstruction algorithm [45]. The resulting 3D data can be digitally sliced and viewed along any angle. Example slices from the three orthogonal axes indicated by the blue, yellow, and green lines in Figure 1A and B are shown in Figure 1B–D. The pixel size and slice thickness of the digital slices is 2 μm. The technique has sufficient resolution to visualize the finest vessel structures, such as those of the perineural vascular plexus, with an estimated diameter of 4 μm (Figure 1E).

Surfaces can be rendered from the 3D data set using an isosurface algorithm (see Materials and Methods) that creates a digital surface corresponding to constant intensity values in the reconstructed data. The resulting 3D surface contains those structures of the vascular network whose intensity values were greater than the chosen isovalue, which was selected to be approximately 1/4 the way between the background and maximum intensity value. The isosurface can be used to visualize the complete vasculature of the embryo as shown in Figure 2A. It can be zoomed in to give a magnified view of structures of interest, as in Figure 2B, and it can be arbitrarily rotated to view the surface from any angle, as illustrated in Figure 2C. The 3D data can also be manually segmented (see Materials and Methods), and rendered in different colours according to the segmentation label.

#### Table 1. Acronyms Used. The list of vessel acronyms used in this paper.

| Acronym | Vessel                                      |
|---------|---------------------------------------------|
| AVS     | Arteriovenous shunt                        |
| ACV     | Anterior cardinal vein                      |
| CCV     | Common cardinal vein                       |
| DA      | Dorsal aorta                               |
| DLAV    | Dorsal longitudinal anastomotical vessel    |
| ICA     | Internal carotid artery                     |
| ISA     | Intersomitic artery                        |
| ISV     | Intersomitic vein                          |
| PCV     | Posterior cardinal vein                    |
| PVH     | Primary head vein                          |
| PMA     | Primitive maxillary artery                 |
| PNVP    | Perineural vascular plexus                 |
| OA      | Omphalomesenteric artery                   |
| OV      | Omphalomesenteric vein                     |
| UA      | Umbilical artery                           |
| UV      | Umbilical vein                             |
| SV      | Sinus venosus                              |
| VTA     | Vertebral artery                           |

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This is demonstrated in Figure 2D which shows a surface rendering of the Cy3-PECAM-1 signal from a 19 somite embryo, with the dorsal aorta (DA), heart and internal carotid arteries (ICA) rendered in yellow, the umbilical vein (UV) in pink and the rest of the Cy3-PECAM-1 signal in blue. Any set of labels can be excluded from the rendering to simplify visualization of structures of interest. The removal of the vasculature in the head, for example, simplifies visualization of the ICA (Figure 2E). Much of the following data is represented in this way.

Volumes can be alternatively rendered from the 3D data set using a direct volume rendering algorithm (see Materials and Methods) that assigns to each voxel arbitrarily selected emissive and absorptive properties according to its reconstructed intensity value and projects an image of the volume onto a 2D image plane. Volume renderings are particularly useful when visualizing co-registered 3D data sets, or when a 3D data set has small features or features with weak intensity values. A rendered volume can be used to visualize the Cy3-PECAM-1 signal and the nonspecific tissue autofluorescence separately (Figure 3A,B respectively) or, as the data sets are co-registered, simultaneously (Figure 3C). This process was performed on all 24 embryos ranging from 5 to 30 somites (Table 2), from which six are shown in Figure 4, to provide a complete map of PECAM-1 expression throughout the entire embryo. Reconstructions from embryos older than 20 somites had insufficient resolution to resolve the finest vascular details in the complete embryo, but were sufficient to position larger vessels and overall structure (data not shown).

These results demonstrate that the FDR-deconvolution OPT technique is capable of resolving the full range of vessels throughout whole embryos spanning the stages of 5–20 somites. Results presented below also illustrate one of the key benefits of the molecular specificity of FDR-deconvolution OPT as compared to imaging based on perfusion of the vascular lumen in that we were able to visualize endothelial cells and vasculature that is disconnected from the rest of the vascular tree and thus identify the origins of vessels at an earlier period of development. FDR-deconvolution OPT is the only imaging technique that has been shown to provide the combination of resolution, specimen
coverage and molecular specificity necessary to image the complete vascular network in whole mouse embryos. Sufficient resolution is made possible in OPT imaging only by the inclusion of FDR-based deconvolution, which was shown to provide a factor of two resolution improvement over standard OPT imaging [41]. Our implementation of FDR-based deconvolution is performed entirely in software and can thus be used in conjunction with any OPT device without the need for hardware modification. FDR-deconvolution OPT is thus particularly well suited to the challenges presented by the developing vascular system of the mouse embryo and would be similarly suitable for imaging other detailed structures such as the developing nervous or lymphatic system.

We present here for the first time, a high-resolution three-dimensional atlas of the developing mouse vasculature in its native state between E8.0 and E10.0 of gestation (5–30 somites). The atlas comprises the collection of all 3D FDR-deconvolution OPT data sets of embryos ranging from 5 to 30 somites, as listed in Table 2. Videos of each of the data sets are made available at http://www.mouseimaging.ca/research/mouse_atlas.html. In the remainder of this paper, we present new information regarding the normal development of mouse vasculature that was obtained from analysis of the embryonic mouse vascular atlas.

Vascular Development Between the 5 and 8 Somite Stage
Continuous PECAM-1 expression in the 5 somite embryo was confined to the completed dorsal aorta (DA), the heart and the allantois as previously reported [2]. Disconnected clusters of PECAM-1 expression were evident throughout the cephalic mesenchyme and lateral mesoderm at this age (Figure 5, Supplemental Video S1). These discrete clusters of PECAM-1 expressing cells were not connected to established vessels, suggesting they were locations of vasculogenesis rather than angiogenesis, consistent with observations that cephalic mesoderm has intrinsic angiogenic potential and contributes to the vasculature of the head [46].

By 7 somites, some PECAM-1 clusters in the cephalic mesoderm had begun to aggregate together forming a single larger vessel predominantly along the anterior-posterior axis and the future location of the primary head vein (PHV), while other cells remained as yet disconnected. By 11 somites virtually all...
PECAM-1 expression in the cephalic mesoderm was connected and formed a rudimentary vascular plexus that lined both the neural tube and the cephalic body wall. Recognizable structures such as the PHV, primitive maxillary artery (PMA) and the primitive internal carotid artery (ICA) were evident at this stage. Left-right communication between the two hemispheres of cephalic plexus was initiated at 7 somites by two vessels originating from the branch point of the PMA and the ICA, extending medially towards each other and forming a complete vessel by 13 somites (data not shown). By 14 somites the smaller vessels lateral to the plexus combined to form the anterior cardinal vein (ACV), as has been previously reported [44]. The plexus had also extended to surround the length of the neural tube and the otic vesicle, and the recognizable pattern of the mesencephalic artery and cephalic veins had begun to emerge in the cephalic body wall. This pattern continued to develop and refine up to the 20 somite stage.

Connection of Embryonic and Extraembryonic Circulation

The extraembryonic circulation of the mouse embryo is divided into two components: the omphalomesenteric (vitelline) vessels and the umbilical vessels. The former connect the embryo proper with the yolk sac, while the latter connect the embryo proper with the feto-maternal interface in the placenta.

The omphalomesenteric artery (OA) connects the yolk sac to the junction of the paired dorsal aorta at the most posterior tip of the embryo at E8.5. After turning, the OA leaves the embryo posterior to the developing heart, and eventually is folded back and fused to the dorsal aorta at E10.5 [47]. The OA has been previously reported to be present in the mouse as early as 7 somites [48]. We observed the OA as a single vessel in the 5 somite embryo that remained throughout the entire range of all embryonic stages studied and had folded back and fused to the DA by 30 somites (Figure 6).

The omphalomesenteric veins (OV) are paired vessels that connect the yolk sac to the sinus venosus (SV) of the embryo. The OV were located posterior to the developing heart throughout all stages imaged, initially draining directly into the SV (Figure 6A). The OV were exclusively extraembryonic until turning, at which point part of the OV traversed the future hepatic location in the embryo proper before connecting to the CCV. We observed the OV to be complete in the 5 somite embryo and present throughout the entire range of all embryonic stages studied (Figure 6).

The umbilical artery (UA) is initiated by de novo vasculogenesis in the allantois at E7.5 [49] and is of particular interest as it is implicated as a site of hematopoietic stem cell development [50,51,52,53,54]. We observed the UA in the 5 somite embryo as a vessel fully formed throughout the allantois but unconnected to

| Som # | 5–6 | 7–8 | 8–9 | 9–10 | 10–11 | 12–13 | 13–14 | 15–16 | 16–17 | 18–19 | 19–20 | 20+ | Total |
|-------|-----|-----|-----|------|-------|-------|-------|-------|-------|-------|-------|-----|-------|
| n     | 3   | 1   | 2   | 2    | 1     | 2     | 1     | 2     | 1     | 2     | 1     | 6   | 24   |

Twenty four (24) embryos were imaged in total across an age range from 5 to 30 somites.

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the dorsal aorta (data not shown). The UA in the allantois fused to the paired dorsal aorta at the base of the allantois by 7 somites (Figure 6A), consistent with previous reports of 6 somites [49], and remained an extraembryonic vessel through the entire range of all embryonic stages studied (Figure 6).

The umbilical vein (UV), unlike the UA, has embryonic as well as extraembryonic components, leading us to question its origins. We were able to trace the origins of the UV back to the 5 somite stage embryo. At this stage it was observed bilaterally as a disconnected string of PECAM-1 expressing cells at the junction of the body wall (ectoderm) and the amnion extending from the sinus venosus (SV) to the posterior tip of the embryo (Figure 7A). The disconnected nature of the Cy3-PECAM-1 signal indicated that the UV is formed by vasculogenesis. These cells then aggregated in a primarily anterior-posterior fashion to extend the length of the embryo (Figure 7B). As the embryo turned, the posterior end of the nearly completed UV was brought into contact with the base of the allantois, thereby allowing connection between the extraembryonic (allantoic) and embryonic portions (Figure 7C). By the end of turning (~14 somites), the remaining cells had joined together to complete the UV as bilateral axial vessels running the length of the trunk from the SV through to the allantois (Figure 7D). At the 7 somite stage, several small branches were observed to extend from the rudimentary UV, which, by the 14 somite stage, developed into a capillary plexus permeating the body wall surrounding the intraembryonic coelom dorsal to the UV (data not shown). This plexus continued to develop along the UV with increasing age, eventually becoming continuous with either the rudimentary PCV or the intersomitic veins (see below).

To our knowledge, this is the first report of how the embryonic portion of the UV is established. The rudiments of this vessel, along with the cephalic plexus described above, were likely present in the embryos examined by Drake and Fleming [2] in their study of vasculogenesis including embryos from 5 to 8 somites, however they would not likely have been recognized as such, in part due to partial dissection of the embryos to facilitate imaging and the limited nature of the 2D confocal imaging used. These findings demonstrate the value of our technique of maintaining the original morphology of the embryo and 3D imaging of developing structures in their entirety.

The 3D nature of FDR-deconvolution OPT data allows for measurements of vascular structures. We measured the relative diameter of the omphalomesenteric and umbilical vessels to serve as an indicator of relative blood flow volume. The diameter of the
UA was less than that of the OA until the 19 somite stage embryo, at which point the two were approximately equal. By 28 somites, the diameter of the UA was approximately 1.5 times that of the OA. The UV, like the UA, were smaller in diameter than the OV until the 19 somite stage embryo, at which point the two were approximately equal. By the 26 somite stage, the UV was found to be approximately 1.5 times the diameter of the UV. Together, these results suggest that the volume of blood flow begins to favour the feto-maternal interface at approximately 20 somites or E9.0–E9.5, consistent with other data showing that the embryo becomes dependent on the chorioallantoic placenta by E10.0 [55].

Intersomitic Vessels of the Occipital Region

Intersomitic (intersegmental) vessels are a useful model for sprouting angiogenesis and vessel pathfinding. They are the first vessels in the embryo to form by sprouting angiogenesis and their navigation between somites is guided by the same cues that guide axon growth cones [reviewed in [56,57,58,59]]. Intersomitic arteries and veins (ISA and ISV) are branches of the DA and PCV respectively that extend dorsally between the borders of their adjacent somites. The development of intersomitic vessels over time can be followed in a single embryo at a single time point as they emerge in a temporally regulated fashion along the anterior-posterior axis from oldest to youngest, similar to their adjacent somites. Comparison of embryos at different stages of development revealed that there was a distinction in the development of intersomitic vessels bounded by the first 5 (occipital) somites compared to those bounded by trunk somites (at least for somites 6–20). As such we refer to these intersomitic vessels as “occipital” and “trunk” respectively.

We observed the occipital intersomitic vessels to consist of three interconnected vessels: a transient ISA, a transient arteriovenous shunt (AVS), and a persistent ISV. The ISAs formed first, initiating bilaterally as dorsal sprouts from the DA as early as 5 somites. At the level of each dorsal ISA, a lateral branch originating from the DA was observed to extend towards the CCV, eventually connecting the two major vessels (Figure 8A). This created a direct communication between the DA and CCV and comprised an AVS. Upon reaching the dorsal margin of the bounding somites, the distal tips of the ISAs branched longitudinally, fusing with the neighboring ISAs to form the vertebral artery (VTA). Shortly after VTA formation, the ISV emerged as a
branch between the CCV and the ISA. We cannot comment on the origin of this vessel as we did not observe any instances when the ISV was connected to solely either the CCV or the ISA. The fusion of the ISV to the ISA created a temporary triangular vascular structure involving the ISA, the AVS and the ISV. This was a short-lived structure, as regression of the ISA and AVS quickly followed (Figure 8B). All ISAs and AVS of the occipital region had regressed by the 18 and 28 somite stage respectively, leaving the CCV connected to the VTA via an ISV and the DA fully separated from the VTA and CCV. Overall, development of the occipital intersomitic vessels was a rapid process as evidenced by the rarity of capture of intermediate developmental stages.

The existence of transient DA-CCV arteriovenous shunts in the development of occipital intersegmental vessels has not been previously reported and it is unclear why they form at all. AVS similar to the DA-CCV shunts have been noted in the course of normal embryonic mouse vascular development connecting the primordial ACV directly to the DA, anterior to the first somite [44]. We were able to confirm the existence of these AVS in the same location in embryos with 8 somites (data not shown). Like the

**Figure 6. Connections between the embryonic and extraembryonic circulation in the early mouse embryo.** (A) At 7 somites, the omphalomesenteric arteries and veins (green) are large structures. The UA (dark pink) connects to the DA and traverses the allantois, but is quite small. The DA and heart is surface rendered red, and the embryo autofluorescence volume rendered with a hot metal colourmap. The development of the UV is shown in Figure 6. (B) At 14 somites, the UA is accompanied by a series of smaller vessels connecting to nearby vasculature. At this point, it is still smaller than the OA and OV. After turning, the omphalomesenteric vessels branch off on one side of the embryo, and the umbilical vessels the other. (C) By 19 somites the UA has grown in size and is approximately equal in diameter to the OA. (D) By 26 somites, the OA is significantly smaller in diameter than the UA, suggesting that the balance of flow begins to favour the feto-maternal interface through the UA at some time between E9.0 and E9.5. All scale bars represent 250 microns.

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Figure 7. The development of the umbilical vein in the mouse embryo. (A) Discrete clusters of PECAM-1 expressing cells (dark pink) were evident along the length of the body wall immediately next to the junction of the body wall to amnion. The DA and heart is surface rendered yellow, and the embryo autofluorescence volume rendered with a hot metal colourmap. (B) The cells aggregated in a primarily anterior-posterior fashion beginning at the SV. (C) By 11 somites, the UV was almost complete, and had begun to develop a plexus which extended dorsolaterally. At the end of turning, the UV was complete and joined to the extraembryonic components of the vessel. (D) By 15 somites the UV is the second largest vessel in the embryo trunk. All scale bars represent 100 microns.

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Figure 8. Development of the occipital intersomitic vessels. (A) The various stages of occipital intersomitic vessel development can be visualized in the 8 somite embryo (inset). The vessels surrounding somites 1 through 5 are segmented as DA (red), intersomitic vessels (pink) and the early cardinal vein (blue). The vessels initiate bilaterally, and an AVS originating from the DA connects to the cardinal vein (yellow arrowheads). An ISV then develops connecting the distal tip of the ISA to the cardinal vein (blue arrowhead). The ISA soon regresses (white arrowhead) leaving the vertebral artery connected to the cardinal vein. Somite number 4 is labelled as S-4. (B) The transient AVS have regressed (yellow arrowheads) by 15 somites, leaving only the expected dorsal ISA (pink), connected by the VTA (green), which connects via ISVs (light blue) to the ACV or CCV (blue). Somites numbers 1 and 4 are labelled as S-1 and S-4. All scale bars represent 100 microns.

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transient AVS between the DA and CCV, the AVS between the DA and the ACV were no longer present beyond the 18 somite stage, and were presumably pruned from the vasculature.

These transient connections, especially those between the DA and CCV, may be causative of the arteriovenous malformations (AVMs) that arise in mice defective for Notch [6,60,61] and TGFβ signalling [62,63]. AVMs are miscommunications between arteries and veins that bypass the normal capillary plexus, resulting in the shunting of blood from the arterial circulation directly back to the venous circulation. Ink flow patterns and histological analysis suggested that AVMs in Notch and TGFβ mutants involved a shunting of blood from the DA into the ACV and CCV [6,60,61,62,63]. Importantly, these AVMs were observed at an age by which transient connections between the DA and the ACV and CCV should have fully regressed, suggesting that the AVMs could have arisen from failed regression of these naturally occurring connections. This would imply that Notch and TGFβ may be required for the coordinated regression of these naturally occurring connections between arterial and venous vessels. Notch and TGFβ may accomplish this through their known role in regulating artery/vein identity [6,60,61,62,63]. Artery/vein identity has been proposed to prevent AVMs either by keeping arterial and venous progenitors separate during vasculogenesis [64] or by preventing “promiscuous fusions between naïve endothelial sprouts” from pre-existing arteries and veins [62]. If we are correct, then artery/vein identity may have a third role: promoting the regression of pre-existing connections between arterial and venous territory. As hemodynamic forces are known to influence vascular remodelling [4,5] it would be necessary to test whether Notch and TGFβ act downstream of blood flow to regulate such remodelling or are part of a true genetically pre-programmed event. Close examination of DA–CCV AVM shunt fate in Notch, TGFβ and blood flow mutant embryos would help shed light on this.

Intersomitic Arteries of the Trunk Region

Intersomitic vessels of the trunk differed from those of the occipital somites, both in their method of formation and final configuration. Trunk intersomitic vessels began to emerge bilaterally at the 8 somite stage as small dorsal protrusions in the paired DA, until the embryo began turning at the 11 somite stage (data not shown). Between this stage and the 12 somite stage (half turn), the protrusion at the level of the second trunk ISA (7th somite) had extended dorsally between the somites, and by 3/4 turning (13 somites), the protrusions down to the 11th somite had become extended branches. Subsequent ISA were observed to extend in more regulated intervals with advancing somite stage (Figure 9). Whereas zebrafish ISAs emerge from the aorta as narrow capillary-like projections composed of 3 or 4 linked endothelial cells [28,65], each ISA in mouse appeared as a sheet-like evagination that was subsequently remodelled into a capillary-like ISA and DLAV (Figure 9). Unlike the occipital ISAs, transient branches directly connecting the DA and PCV were not observed,

Figure 9. Development of the cervical intersomitic vessels. (A) The various stages of cervical intersomitic vessel development can be segmented as visualized by surface renderings in the 16 somite mouse embryo. The vessels along the right side of the embryo and surrounding somites 1 through 16 are labelled as: DA (red), ISA (pink), ISV (blue), VTA, DLAV and PNVP (green), ACV and CCV (cyan), UV (dark pink), UV plexus (purple), and PCV (blue). Somites 1, 5, 10 and 15 are numbered as S-1, S-5, S-10 and S-15. (B) Branches of PECAM-1 expression originating from the tips of the ISAs (yellow arrowheads) were observed to turn towards the location of the future PCV. (C) A second branch from the ISA was also observed to extend in a predominantly anterior direction (pink arrowheads) to connect up with other ISAs, eventually forming the DLAV. PECAM-1 expression along the location of the expected PCV was observed to lag development of the ISAs and is discontinuous (yellow arrowheads). (D) The PNVP develops through remodelling of the VTA and DLAV. Branches initiate medially from the DLAV (pink arrowhead), begin to remodel into simple mesh (blue arrowhead), and eventually remodel into a fine structured capillary plexus surrounding the neural tube. Note at this stage that the first ISA has regressed. Scale bars represent 100 microns.

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and ISAs were not seen to regress at any stage up to 30 somites. Distinct mechanisms of intersomitic vessel development in the occipital and trunk regions are fully consistent with differences in somitogenesis in these two regions. Unlike trunk somites, occipital somites do not form from presomitic mesoderm using the segmentation clock mechanism [66]. Furthermore, unlike trunk somites, occipital somites disperse and become undetectable as segments soon after their formation. As endothelial cells are highly responsive to their local microenvironment [67], differential patterning of occipital and trunk somites may explain why intersomitic vessels in the occipital region differ to those of the trunk.

Intersomitic Veins and the Posterior Cardinal Vein

As the ISA approached the dorsal boundary of the somites, a branch was observed to extend laterally from the ISA toward the location of the future PCV, which is lateral to the DA (Figure 9B). This lateral branch became progressively more defined with age and formed the rudiment of the ISV, eventually connecting to either a rudimentary PCV or directly to the capillary plexus from the UV. A small number of branches of PECAM-1 expression were also observed to extend from the UV capillary plexus or the rudimentary PCV towards the rudimentary ISV. As for occipital ISA, each trunk ISA was paired with an accompanying ISV. This arrangement is in contrast to zebrafish, where the ISV sprouts from the PCV to join the ISA and in response to hemodynamic pressure, an alternating pattern of ISA/ISV is established [28].

PECAM-1 expression in the region of the expected PCV was first noted at 15 somites extending posteriorly from the CGV and connecting to the ISVs in a predominantly anterior-posterior fashion (Figure 9). Discontinuous PECAM-1 expression was observed along the path of the future PCV strongly suggesting its formation was a vasculogenetic process, as occurs in the chick and zebrafish [29,68]. Branches extended ventrally from the PCV and became continuous with the UV plexus as described above. By 16 somites PECAM-1 expression was continuous to the 8th ISV, extending discontinuously down to the 15th ISV, and by 19 somites the expression was continuous through all ISV. The vasculogenic activity in the vicinity of the PCV and its juxtaposition to the newly emerging ISVs makes it difficult to determine whether the ISV truly sprouts from its accompanying or is generated by (PECAM-1 negative) angioblasts recently added to the PCV by vasculogenesis. Detailed analysis of Flk1 expression as a marker of immature angioblasts combined with live imaging studies will be needed to resolve this issue.

PCV formation was disrupted in the vicinity of the developing limb bud (somites 8/9–13/14 [69]). At the 14 somite stage, an AVS connecting the DA to the rudimentary PCV was observed at the location of the 13th ISA, immediately posterior to the developing limb bud. This vessel was observed to develop bilaterally, but not necessarily at the same somite level. At the 20 somite stage, this vessel was still present in the same location, and at the 25 somite stage it had developed into an artery feeding the developing limb. Additional branches were noted at this stage, originating from the DA and the surrounding ISAs and connecting to the capillary plexus of the developing limb bud. The forelimb field, as defined by Tbx5 expression in lateral plate mesoderm, is first evident at the 8 somite stage [70]. We noted the limb bud itself to begin its formation at approximately 12 somites, and spanned the 8th to the 12th ISA. Together, these results suggest that the appearance of these limb arteries lags the development of the limb bud, and thus may be in response to a changing environment rather than a programmatic or preemptive occurrence.

Anterior Branching of Intersomitic Arteries Establishes the DLAV

Upon reaching the dorsal margin of the somite and after formation of an ISV bud, trunk ISA tips branched longitudinally and fused with their adjacent ISAs forming the DLAV. This longitudinal branching was strongly biased in an anterior direction suggesting that it was guided by an attractive or repulsive mechanism (Figure 9C). The anterior bias of dorsal ISA branchings was not absolute, as some dorsal branches were seen to extend in a posterior direction, and in some cases bidirectionally. From 42 trunk ISAs for which branching directionality could be demonstrated, the number of anterior/posterior/bidirectional branching was 36:4:2, suggesting a strong bias for anterior branching of trunk ISAs. In the occipital region, ISAs observed were either completely connected to the VTA or had not yet begun to branch, thus we were unable to determine whether bias exists in occipital ISA branching. We observed instances of some trunk ISAs reaching the dorsal somite margin and initiating longitudinal branching prior to ISAs located anteriorly, indicating that timing of ISA remodelling is not absolute and is a highly dynamic process.

Anterior bias of trunk ISA branching is in contrast to the zebrafish, where ISAs branch in both anterior and posterior directions after reaching the dorsal boundary of the somite [28]. As described above, intersomitic vessel branching and pathfinding are regulated by the same guidance cues used by the axon growth cone. Somites are divided into rostral and caudal halves and many genes associated with axon guidance show polarized expression in these halves [71]. An anterior bias of ISA branching could be explained by attractive cues in the caudal half-somite anterior of the sprout or repulsive cues in the rostral half-somite posterior to the sprout.

EphrinB2 is one possible ISA attractant. In addition to being expressed in the ISA itself, ephrinB2 is expressed in the caudal half-somite during the time that longitudinal ISA branching is occurring [44,72]. In vitro, ephrinB2 can induce endothelial sprouting [72,73]. In vivo, ephrinB2+/− mice show defective vascular sprouting into the CNS [74] and reduced lymphatic sprouting [75], consistent with an attractive role for ephrinB2. While ephrinB2−/− mice display defective intersomitic vessel patterning that can be attributed to vascular specific ephrinB2 [44], these mutants were assessed after the DLAV had formed. Analysis of earlier time points in ephrinB2+/− embryos or ideally a somite specific ephrinB2 knockout would need to be performed to determine whether ephrinB2 is required for the anterior branching bias.

Our observations of preferential anterior branching toward the caudal half-somite would seem inconsistent with the expression of a known repulsive vascular cue, Sema3E, in this part of the somite [76]. One possibility is that Sema3E is not expressed early enough in the caudal half-somite to repel initial dorsal branching of the ISA, which we observed to occur before somites disperse into sclerotome and dermamyotome. Sema3E expression in the caudal half-somite and defects in intersomitic vessel branching were reported at E10.5 and E11.5, after somites have begun to disperse and form sclerotome and dermamyotome compartments [76,77]. Sema3E may therefore affect intersomitic branching that occurs subsequent to the primary branching we describe here. Similarly, the restriction of neural crest migration and peripheral nervous system axon pathfinding to the rostral half-somite occurs after formation of the sclerotome [71,78,79]. Guidance cues active in somites at this time such as the anti-angiogenic thrombospondin-1 in the rostral half-somite [80] are therefore not likely to be relevant to the vascular guidance we observed. Whatever the mechanisms
are that govern the guidance of this branch, they are likely to involve a complex interplay of multiple attractive and repulsive factors, which together provide an anterior bias.

Formation of the Perineural Vascular Plexus

The perineural vascular plexus (PNVP) is the precursor to the blood brain barrier and is recruited to surround the neural tube in response to VEGF between E6.5 and E9.5 [81,82]. In mouse, vascular sprouts from the PNVP invade the neuroepithelium around E10.0 in a stereotypic fashion [83,84,85]. PNVP sprouting into the neuroepithelium is mediated by VEGF [86], ephrinB2 [74] and Tie1 [67], while subsequent branching and remodelling of the sprouts in the neuroepithelium is regulated by Np1 [85], heparin-binding VEGF isoforms [86,88], Dll4/Notch [43] and netrin1/UnC5b [89] signalling. Failure of the PNVP to invade the neuroepithelium results in neurodegeneration and neonatal lethality demonstrating the importance of this plexus to organogenesis [86].

The perineurial vascular plexus is the precursor to the blood brain barrier and is recruited to surround the neural tube in response to VEGF between E6.5 and E9.5 [81,82]. The PNVP extends as a capillary plexus around the neural tube at the level of the first somite. The PNVP extends into the peripheral somite region as a capillary plexus extending ventrally from the VTA and then at the 16 somite stage as a capillary plexus extending from the VTA and extending ventrally from the VTA and then at the 16 somite stage. This appearance of the rudimentary branches occurred soon after fusion of the VTA between two adjacent ISAs. The PNVP extends to the 8th somite at the 20 somite stage, and down to the 20th somite by the 30 somite stage. Consistent with previous reports, the PNVP was first observed to invade the neural tube at the 27 somite stage (data not shown) [83,84].

Quail-chick and mouse-quail chimera studies have shown that somites [81] and references therein) and lateral mesoderm (81) and references therein) are major sources of PNVP endothelial cells in the trunk. The fine chimerism between host and graft derived cells in the PNVP led the authors of one study to conclude that somite derived angioblasts migrated to and incorporated into the PNVP as they have been demonstrated to do in the chick DA [92,93], or, alternatively, somite derived vascular cells in the body wall may simply fuse to the pre-existing PNVP and contribute to it in that way.

Conclusion

We have employed FDR-deconvolution OPT to generate a high-resolution three-dimensional atlas of the developing mouse vasculature in its native state between E8.0 and E10.0 of gestation (5–30 somites). Analysis of the 3D reference atlas we have constructed has revealed significant new information regarding normal development of the embryonic mouse vasculature. The need for an atlas such as this is critical, as numerous pathways required for vascular development exhibit severe vascular phenotypes during this time period when disrupted. This atlas can thus be used as a tool for better interpretation of these vascular phenotypes and as a platform to provide insight into normal mammalian vascular development. The observations in this paper represent only a portion of the information available in this atlas, which is provided for further study at http://www.mouseimaging.ca/research/mouse_atlas.html.

Materials and Methods

Embryo collection and Staining

Wild type ICR embryos were collected between the ages of embryonic day (E) 8.0 (5 somites) and E10.0 (30 somites). Noon of the plug day was considered to be E0.5. Embryos were dissected from their decidua and Reichert’s membranes, then, to maintain natural shape, were fixed for 1 h in 4% paraformaldehyde before remaining extraembryonic tissues were removed. For incompletely turned embryos, the amnion and the portion of yolk sac contiguous with the embryo were left attached to prevent disruption of embryonic-extraembryonic circulation. Embryos were then dehydrated through a graded series of methanol (25%, 50%, 75%, 100%) and stored at −20°C. Before staining, embryos were rehydrated and endogenous peroxidase activity was quenched with 3% H2O2. Non-specific antibody binding was blocked by pre-incubating embryos in 1% heat inactivated FCS (Hyclone, Logan UT) and 1% normal goat serum (Cedarlane, Burlington ON). Embryos were then stained overnight with 5 μg/mL anti-PECAM-1 antibody (Mec13.3) (BD Pharmingen).

Primary antibody was detected by staining overnight with anti-rat HRP secondary antibody (Biosource, Camarillo CA) followed by incubation with tyramide-Cy3 reagent (1:50) for 1 h (PerkinElmer, Boston MA). Experiments were approved by the Animal Care Committee of Mount Sinai Hospital (Toronto, ON, Canada) and were conducted in accordance with guidelines established by the Canadian Council on Animal Care.

Although all embryos presented in this study were processed according to the above protocol, we have since determined that methanol fixation slightly decreased the signal to noise ratio. While this effect did not significantly affect our imaging or findings, we would recommend replacing methanol fixation with a longer (4 h) paraformaldehyde fixation time followed by treatment with 50 mM sodium azide prior to quenching endogenous peroxidase by H2O2 treatment. A detailed description of the protocol is available at http://www.sickkids.ca/rossant/custom/protocols.asp.

Optical Projection Tomography (OPT) of embryos

Optical projection tomography was performed as described previously [41]. Specimens were embedded in 1% low melting point (LMP) agarose and subsequently cleared using a 1:2 mixture of benzyl alcohol and benzyl benzoate (BABB). The index-matched specimen was suspended from a stepper motor and immersed in a BABB bath with optically flat parallel glass windows. Images of the specimen were formed using a Leica MZFLIII stereozoom microscope equipped with a 0.5× objective lens and a 1.0× camera lens. Typical zoom settings used for image formation were between 4.0×–6.3×, resulting in numerical apertures from 0.0465 to 0.0620. Images (termed slices) were acquired with a Retiga Es1 CCD camera with pixel size

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6.45 \times 6.45 \text{ microns}. Light from a mercury lamp was directed onto the specimen and filter sets were used to create fluorescent images of the specimen. An autofluorescence view was captured with the GFP1 filter set in the illumination and detection light path, and a view of the Cy3 fluorescence from the specimen was captured using the Cy3 filter set in the illumination and detection light path. The sample was rotated stepwise with a 0.9° step size through a complete revolution and views were acquired at each step.

Each OPT view approximates a parallel ray projection through the specimen. The temporal sequence from a row of detectors on the CCD forms a sinogram that is used to reconstruct the corresponding slice through the specimen using the standard convolution filtered back-projection algorithm [45]. The reconstruction of all slices yielded a 3D volumetric representation of the specimen. The stack of Cy3 views from a single specimen were subjected to Frequency Distance Relationship (FDR)-based filtering as described below, and both the filtered and unfiltered views reconstructed separately. The resulting 3D reconstruction of autofluorescence views and its corresponding 3D reconstruction of the specimen. The stack of Cy3 views acquired over a complete revolution and views were acquired at each step. An isolated bead was found in the stack. The data was resampled to approximate the PSF of the system at a focal plane through the specimen, and an image of the bead plug was acquired at each step.

The resolution achieved was estimated to be 5 microns at the floor and the high threshold chosen to maximize vessel visibility. Reconstructions were segmented using the Amira module for visualization. Surface renderings were created using the Amira “isosurface” module with a threshold chosen just above the noise floor. Volume renderings were created using the Amira “Voxel” module, with the low threshold being chosen just above the noise floor and the high threshold chosen to maximize vessel visibility. Volume renderings of the autofluorescence reconstruction overlapping the Cy3-PECAM-1 reconstruction were created using a red colourmap for the autofluorescence and a hot metal colourmap for the Cy3-PECAM-1.

Reconstructions were segmented using the Amira module “LabelField” to maximize vessel visibility and aid in image interpretation. Observations were confirmed in both the unfiltered and filtered Cy3-PECAM-1 reconstructions. Surface renderings of the segmented vessels were performed using the same threshold but different colourmaps.

**Supporting Information**

**Video S1** Cephalic plexus of the 11 somite embryo. Larger vessels such as the PMA (blue) and the ICA (green) are easily distinguishable from the smaller vessels using the red colourmap. Simultaneous visualization of the segmented vessels with the corresponding volume renderings of the same threshold but different colourmaps for the Cy3-PECAM-1 and autofluorescence reveals that the larger vessels are located more superficially. A roll-off filter was used to deemphasize noise, and the PMA filter was applied to the volume renderings. The resolution achieved was estimated to be 5 microns at the floor and the high threshold chosen to maximize vessel visibility. Reconstructions were segmented using the Amira module “isosurface” module with a threshold chosen just above the noise floor. Volume renderings were created using the Amira “Voxel” module, with the low threshold being chosen just above the noise floor and the high threshold chosen to maximize vessel visibility. Volume renderings of the autofluorescence reconstruction overlapping the Cy3-PECAM-1 reconstruction were created using a red colourmap for the autofluorescence and a hot metal colourmap for the Cy3-PECAM-1.
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Author Contributions

Conceived and designed the experiments: JRW RMH. Performed the experiments: JRW LC. Analyzed the data: JRW LC. Wrote the paper: JRW LC JR RMH.

References

1. Ema M, Takahashi S, Rossant J (2006) Deletion of the selection cassette, but not cis-acting elements, in targeted Flk1-lacZ allele reveals Flk1 expression in multipotent mesodermal progenitors. Blood 107: 111–117.
2. Drake CJ, Fleming PA (2000) Vasculogenesis in the day 6.5 to 9.5 mouse embryo. Dev Biol 226: 1071–1079.
3. Huber TL, Kouskoff V, Fehling HJ, Palis J, Keller G (2004) Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. Nature 434: 625–630.
4. Le Moyon D, Pardanaud L, Yuan L, Dyono V, et al. (2004) Flow regulates arterio-venous differentiation in the chick embryo yolk sac. Development 131: 361–375.
5. Lucitti JL, Jones EAV, Huang CQ, Chen J, Fraser SE, et al. (2007) Vascular remodeling of the mouse yolk sac requires hemodynamic force. Development 134: 3317–3326.
6. Gridley T (2007) Notch signaling in vascular development and physiology. Development 134: 2709–2716.
7. Hofmann JJ, Iruela-Arispe ML (2007) Notch signaling in blood vessels - Who is talking to whom about what? Curr Res 100: 1556–1568.
8. Niessen K, Karsans A (2007) Notch signaling in the developing cardiovascular system. Am J Physiol Cell Physiol 293: 1–11.
9. Lebre F, Decker M, Bortolotto P, Ten Dijke P (2005) TGF-beta receptor function in the endothelium. Cardiovasc Res 65: 599–608.
10. Rossant J, Howard L (2002) Signaling pathways in vascular development. Annu Rev Cell Biol 18: 511–537.
11. Astorga J, Carlsson P (2007) Hedgehog induction of murine vasculogenesis is mediated by Foxf1 and Bmp4. Development 134: 3753–3761.
12. Byrd N, Becker S, Maye P, Narasimhaiah R, St-Jacques B, et al. (2002) Hedgehog is required for murine yolk sac angiogenesis. Development 129: 361–372.
13. Vokes SA, Yatskievych TA, Heimark RL, McMahon J, McMahon AP, et al. (2004) Hedgehog signaling is essential for endothelial tube formation during vasculogenesis. Development 131: 4371–4380.
14. Coultas L, Chavengsaksophak K, Rossant J (2005) Endothelial cells and VEGF in vascular development. Nature 438: 937–945.
15. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, et al. (1996) Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 380: 435–439.
16. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, et al. (1996) Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 380: 439–442.
17. Kuijper S, Turner CJ, Adams KH (2007) Regulation of angiogenesis by Eph-ephrin interactions. Trends Cardiovasc Med 17: 145–151.
18. Thurston G (2003) Role of angiopoietins and tie receptor tyrosine kinases in angiogenesis and lymphangiogenesis. Cell Tissue Res 314: 61–68.
19. Pola R, Ling LE, Silver M, Coebley MJ, Kearney M, et al. (2001) The endothelial cell is an indirect angiogenic agent upregulating two families of angiogenic growth factors. Nat Med 7: 706–711.
20. White AC, Lavine KJ, Ornitz DM (2007) FGFR9 and SHH regulate mesenchymal Vegfa expression and development of the pulmonary capillary network. Development 134: 3743–3752.
21. Lee S, Chen TT, Barber CL, Jordan MC, Murdock J, et al. (2007) Autocrine VEGF signaling through Flk1 regulates formation of tip cells during angiogenesis. Nature 455: 776–780.
22. Lobov IB, Renard RA, Papadopoulos N, Gale NW, Thurston G, et al. (2007) Delta-like ligand 4 (Dll4) is induced by VEGF as a negative regulator of angiogenic sprouting. Dev Biol 306: 3219–3224.
23. Igoa S, Hiruguchi S, Weinstein BM (2001) The vascular anatomy of the developing zebrafish: an atlas of embryonic and early larval development. Dev Biol 250: 278–301.
54. Ottersbach K, Dzierzak E (2005) The murine placenta contains hematopoietic stem cells within the vascular labyrinth region. Dev Cell 8: 377–387.
55. Ma J, Adamson SL (2006) Developmental changes in hemodynamics of uterine artery, utero- and umbilical-placental, and vitelline circulations in mouse throughout gestation. Am J Physiol Heart Circ Physiol 291: H1421–1428.
56. Eichmann A, Makinen T, Altaloo K (2005) Neural guidance molecules regulate vascular remodeling and vessel navigation. Genes Dev 19: 1013–1021.
57. Carmeliet P, Tesmer-Lavigne M (2005) Common mechanisms of nerve and blood vessel wiring. Nature 436: 193–200.
58. Suchting S, Bicknell R, Eichmann A (2006) Neuronal clues to vascular guidance. Exp Cell Res 312: 668–675.
59. Jones C, Li DY (2007) Common cues regulate neural and vascular patterning.
60. Suchting S, Bicknell R, Eichmann A (2006) Neuronal guidance molecules regulate vascular remodeling and vessel navigation. Genes Dev 19: 1013–1021.
61. Duarte A, Hirasima M, Beneforto R, Trindade A, Diniz P, et al. (2004) Dosage-sensitive requirement for mouse dfrl in artery development. Genes Dev 18: 2474–2478.
62. Sorensen LK, Brooke BS, Li DY, Urness LD (2003) Loss of distinct arterial and venous boundaries in mice lacking endoglin and a vascular-specific TGF-β1 receptor. Dev Biol 261: 235–250.
63. Urness LD, Sorensen LK, Li DY (2000) Arteriovenous malformations in mice lacking activin receptor-like kinase-1. Nat Genet 26: 328–331.
64. Lawson ND, Weiner BM (2002) Arteries and veins: making a difference with zebrafish. Nat Rev Genet 3: 674–682.
65. Childs S, Chen JN, Garrity DM, Fishman MC (2002) Patterning of angiogenesis in the zebrafish embryo. Development 129: 973–982.
66. Dale K, Pourquié O (2000) A clockwork somite. Bioessays 22: 72–83.
67. Nikolova G, Lammert E (2003) Interdependent development of blood vessels derived angioblasts. Dev Biol 234: 352–364.
68. Zhong TP, Childs S, Chen JN, Garrity DF, Fishman MC (2002) Patterning of angiogenesis in the zebrafish embryo. Development 129: 973–982.
69. Burke AC, Nelson CE, Morgan BA, Tannahill D, Cook GM, Keynes RJ (2004) Somite polarity and organ systems. Cell Tissue Res 314: 33–42.
70. Agarwal P, Wylie JN, Galceran J, Arthikutto O, Li C, et al. (2003) Thrombomodulin is essential for forelimb bud initiation following patterning of the limb field in the mouse embryo. Development 130: 623–633.
71. Kuan CY, Tannahill D, Cook GM, Keynes RJ (2004) Somite polarity and segmental patterning of the peripheral nervous system. Mech Dev 121: 1055–1068.
72. Adams RH, Wilkinson GA, Weiss C, Diella F, Gale N, et al. (1999) Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis, and sprouting angiogenesis. Genes Dev 13: 295–306.
73. Zhang XQ, Takeda N, Okie Y, Inada T, Gale N, et al. (2001) Stromal cells expressing ephrin-b2 promote the growth and sprouting of ephrin-b2(+ve) endothelial cells. Blood 98: 1028–1037.
74. Wang HJ, Chen ZP, Anderson DJ (1998) Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-b2 and its receptor eph-b4. Cell 93: 741–753.
75. Makinen T, Adams RH, Bailey J, Lu Q, Ziemiecki A, et al. (2005) Pdz interaction site in ephrinb2 is required for the remodeling of lymphatic vasculature. Genes Dev 19: 397–410.
76. Gu C, Yoshida Y, Lier J, Reimert DV, Mann F, et al. (2003) Semaphorin 3e and plexin-d1 control vascular pattern independently of neuropilins. Science 307: 265–268.
77. Kaufman MH, Bard JBL (1999) The Anatomical Basis of Mouse Development. Academic Press.
78. Krieger CE (2001) Segmental organization of neural crest migration. Mech Dev 105: 37–45.
79. Sauka-Spengler T, Bronner-Fraser M (2006) Development and evolution of the migratory neural crest: a gene regulatory perspective. Curr Opin Genet Dev 16: 360–366.
80. Tucker RP, Hagiwara C, Chiquet-Ehrismann R, Lavender J, Hall RJ, et al. (1999) Thrombospondin-1 and neural crest cell migration. Dev Dyn 214: 312–322.
81. Hogan BA, Ambler GA, Chapman DL, Bautch VL (2004) The neural tube pattern vessels developentially using the vegf signaling pathway. Development 131: 1503–1513.
82. Evans HM (1969) On the development of the aorta and cardiac and umbilical veins and and the other blood vessels of vertebrate embryos from capillaries. Anat Rec 3: 498–519.
83. Nagase T, Nagase M, Yoshimura K, Fujita T, Koshima I (2005) Angiogenesis within the developing mouse neural tube is dependent on sonic hedgehog signaling: possible roles of motor neuron. Genes Cells 10: 595–604.
84. Nakao T, Ishizawa A, Ogaswa R (1988) Observation of vascularization in the spinal-cord of mouse embryos, with special reference to development of boundary membranes and perivascular spaces. Anat Rec 2: 663–677.
85. Gerhardt H, Ruhrberg C, Abrahamsson A, Fujisawa H, Shima D, et al. (2004) Neuropilin-1 is required for endothelial tip cell guidance in the developing central nervous system. Dev Dyn 231: 505–509.
86. Haigh JJ, Morelli PI, Gerhardt H, Haigh K, Tien J, et al. (2003) Cortical and retinal defects caused by dosage-dependent reductions in vegf-a paracrine signaling. Dev Biol 262: 225–241.
87. Sato TN, Tsuzawa Y, Deutsch U, Wolburg-Buchholz K, Fujisawa Y, et al. (1995) Distinct roles of the receptor tyrosine kinases tie-1 and tie-2 in blood vessel formation. Nature 376: 70–74.
88. Ruhrberg C, Gerhardt H, Haigh K, Watson R, Luminidis S, et al. (2002) Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. Genes Dev 16: 2634–2650.
89. Lu X, Le Noble F, Yuan L, Jiang Q, De Lafarge R, et al. (2004) The netrin receptor unc5b mediates guidance events controlling morphogenesis of the vascular system. Nature 432: 179–186.
90. Ambler CA, Nowicki JL, Burke AC, Bautch VL (2001) Assembly of trunk and limb blood vessels involves extensive migration and vascularization of somite-derived angioblasts. Dev Biol 234: 352–364.
91. Wilting J, Brand-Saberi B, Huang R, Zhi Q, Kontges G, et al. (1995) Angiogenic potential of the avian somite. Dev Dyn 202: 163–171.
92. Pardanaud L, Luton D, Prigent M, Bourcheix L, Catala M, et al. (1996) Two homologous genes in zebrafish. Nat Rev Genet 3: 674–682.
93. Zhang XQ, Takakura N, Oike Y, Inada T, Gale N, et al. (2001) Stromal cells expressing ephrin-b2 promote the growth and sprouting of ephrin-b2(+ve) endothelial cells. Blood 98: 1028–1037.
94. Lu X, Le Noble F, Yuan L, Jiang Q, De Lafarge R, et al. (2004) The netrin receptor unc5b mediates guidance events controlling morphogenesis of the vascular system. Nature 432: 179–186.
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