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Review

Vascular morphogenesis in the zebrafish embryo

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ABSTRACT

During embryonic development, the vertebrate vasculature is undergoing vast growth and remodeling. Blood vessels can be formed by a wide spectrum of different morphogenetic mechanisms, such as budding, cord hollowing, cell hollowing, cell wrapping and intussusception. Here, we describe the vascular morphogenesis that occurs in the early zebrafish embryo. We discuss the diversity of morphogenetic mechanisms that contribute to vessel assembly, angiogenic sprouting and tube formation in different blood vessels and how some of these complex cell behaviors are regulated by molecular pathways.

Introduction

Branched tubular organs, such as the insect tracheal system or the vertebrate cardiovascular system, kidney or lung, are found throughout the animal kingdom. Formation of such tubular networks from precursor cells or tissues involves a variety of morphogenetic processes, such as tube formation, elongation, branching and fusion. These processes are brought about by complex cellular behaviors, which include cell polarization, cell migration, cell rearrangements, cell shape changes and cell division. Although tubular organs are extremely diverse in anatomy and function, the cellular activities that govern tube formation and branching morphogenesis appear to be quite similar (Baer et al., 2009; Andrew and Ewald, 2010). In this review, we describe the current understanding of blood vessel formation in the early zebrafish embryo. We are placing special emphasis on the morphogenetic processes that contribute to vascular development and discuss the regulatory components that accompany these events.

In vertebrates, the cardiovascular system constitutes a highly ramified network of tubes that transports gas, nutrients, hormones and metabolites throughout the body. It also has important roles in the regulation of homeostasis and wound healing and is involved in the pathology of numerous diseases including cancer and inflammation (Carmeliet, 2003). The cardiovascular system emerges as one of the first organs during embryonic development and retains morphogenetic plasticity in adult life. Blood vessels are an integral component of all organs and are vital not only for their function but also for their formation during embryonic development (Nikolova and Lammert, 2003; Red-Horse et al., 2007; Sakaguchi et al., 2008). Blood vessels are highly diverse: they differ in size and are specialized depending on their function and the tissue or organ they are embedded in (Aird, 2007; Rocha and Adams, 2009). In general, they consist of an inner epithelium (endothelium) lining the lumen; depending on the type of vessel, this endothelium is surrounded by a basal lamina and by mural cells, such as pericytes and smooth muscle cells, which both support and regulate the function of the endothelium (Armulik et al., 2005).

Over the last decade, the molecular pathways controlling vascular development have attracted much attention, and a large number of key molecules has been identified that regulate different aspects of blood vessel morphogenesis. The basic frameworks of the vascular anatomy are conserved among vertebrates, which makes it possible to assign homologies between distinct blood vessels and to directly compare the formation of these vessels in different vertebrate species (Isogai et al., 2001; see Fig. 1). The zebrafish embryo has proven to be a useful model to study vascular morphogenesis in vivo. The vasculature can be easily visualized using a variety of labeling techniques, such as endothelial specific expression of fluorescent protein or by microangiography (Fig. 1). Its small size, experimental accessibility, optical clarity and rapid development allow to observe cellular activities, such as cell migration, cellular rearrangements and cell divisions, as they occur during blood vessel formation in the embryo. It is also possible to follow cardiovascular mutant phenotypes for several days because oxygenation of the early zebrafish embryo does

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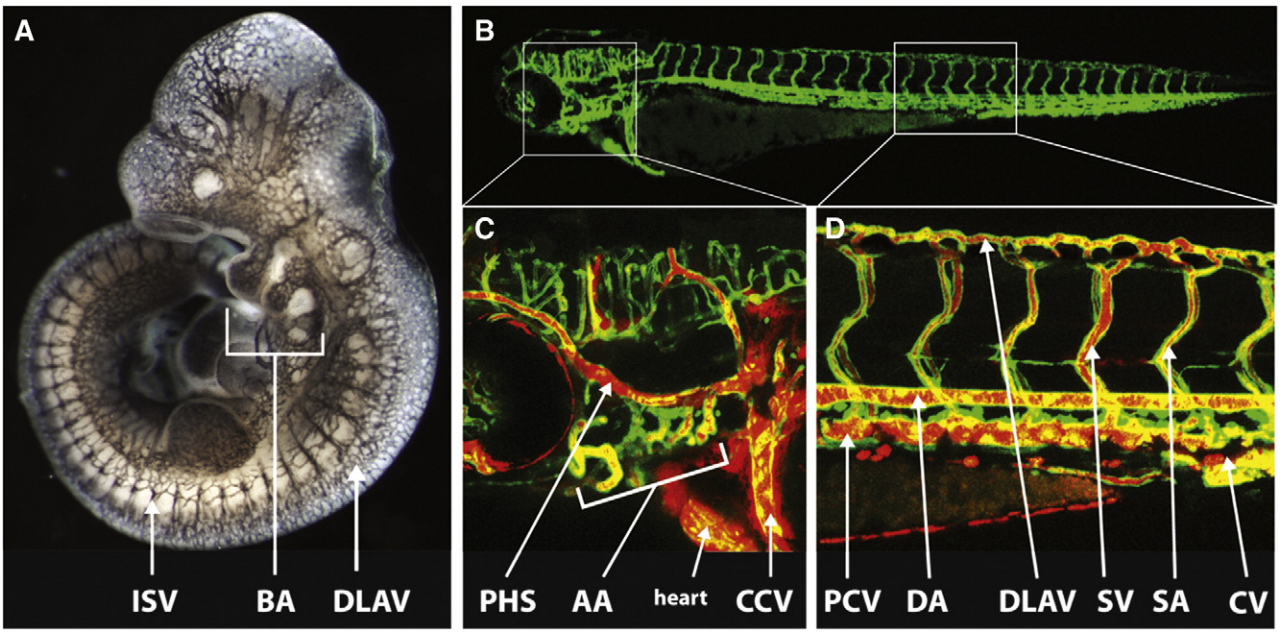

Fig. 1. The vascular system in mouse and fish embryos. (A) Visualization of the vascular system by immunohistochemical localization of PECAM-1 in a day 10 mouse embryo (photo courtesy of Ralf Adams, MPI, Münster, Germany). Owing to the opacity of the mouse embryo, only superficial blood vessels can be seen. BA: branchial arches (1st and 2nd); ISV: intersegmental vessel; DLAV: dorsal longitudinal anastomotic vessel. (B–D) The vascular system in a 3-day-old zebrafish embryo visualized by reporter gene analysis (*TG:flk1:EGFP* in green) and by microangiography using quantum dots (red in panels C and D). Some blood vessels are indicated according to Isogai et al. (2001). AA: aortic arches (1–6); CV: caudal vein; CCV: common cardinal vein; DA: dorsal aorta; PCV: posterior cardinal vein; PHS: primary head sinus; SA: segmental artery; SV segmental vein. At these stages, anatomical similarities between the two species are best observed in the branchial arches and in the ISV of the trunk. ISV and DLAV form quite similarly in both species (Isogai et al., 2003; Walls et al., 2008).

not rely on blood circulation. Furthermore, functional studies by forward and reverse genetics have shown that the molecular components that regulate vascular development are conserved between mammals and fish (Beis and Stainier, 2006; Lawson and Weinstein, 2002b; Thisse, 2002). Thus, the zebrafish embryo presents a unique system in which live imaging can be combined with functional studies to gain a more complete insight into how the molecular and morphogenetic mechanisms are integrated at the (sub)cellular level to shape the vascular tree.

Vasculogenesis

The formation of vertebrate blood vessels is commonly subdivided into two distinct morphogenetic processes, called vasculogenesis and angiogenesis. Vasculogenesis is defined by in situ aggregation of angioblasts into a blood vessel (Coffin and Poole, 1988; Poole and Coffin, 1989; Risau, 1995; Risau et al., 1988), while further sprouting of vessels from existing vessels occurs via a process called angiogenesis (Risau, 1995).

Origin and specification of endothelial cells

Angioblasts are precursors of endothelial cells not yet incorporated into blood vessels. They originate from the ventrolateral mesoderm (Kimmel et al., 1995; Stainier et al., 1995). Analyses of genes expressed in the hematopoietic and endothelial cell lineages have revealed a remarkable conservation between vertebrate species. In particular, transcription factors belonging to the ETS, GATA and LMO families have been shown to control specification of these lineages in mammals as well as fish (De Val et al., 2008; Detrich et al., 1995; Liu and Patient, 2008; Thompson et al., 1998; Zon et al., 1991). At the beginning of somitogenesis, transcription factors, such as *scl*/*tal1* and *lmo2*, which specify angioblasts and hematopoietic cells, are expressed in two domains along the body axis, the anterior and the posterior lateral mesoderm (Dooley et al., 2005; Liao et al., 1998; Patterson et al., 2007). During somitogenesis these cell populations acquire unique gene expression profiles. For example, *flk1*-positive/*scl*-positive precursor cells differentiate into *flk1*-positive/scl-negative and *flk1*-negative/*scl*-positive cells, which will give rise to endothelial and hematopoietic cells, respectively (Gering et al., 1998). There seems to be no transcriptional factor regulating exclusively the endothelial specification but a combination of multiple factors with overlapping expression patterns (reviewed by De Val and Black, 2009).

Formation of the dorsal aorta and the cardinal vein

The basic anatomy of the initial embryonic circulatory system is quite similar among vertebrates. In addition, the first embryonic vessels to appear, the dorsal aorta (DA) and the posterior cardinal vein (PCV), are formed by a distinct morphogenetic mechanism called vasculogenesis in all vertebrates (Isogai et al., 2001). In zebrafish, angioblasts are specified well before the first blood vessels are formed. Expression of molecular markers such as *fli1a* shows that angioblasts are located in two lateral stripes at 12–14 hpf. By 28–30 hpf, the DA and the PVC can be discerned and are fully lumenized (Roman et al., 2002). In vivo imaging, using a *Tg(fli1a:EGFP)* reporter fish line, has shown that angioblasts migrate as individual cells towards the embryonic midline where they coalesce (Lawson and Weinstein, 2002b). During recent years, a considerable amount of research has focused on how this migration process is regulated, how these cells form the axial vessels and how DA and PCV are specified. As indicated in Fig. 2, the PCV forms subsequently to the DA (Eriksson and Löfberg, 2000; Herbert et al., 2009; Jin et al., 2005), and this relationship appears to be conserved among vertebrates (Coffin and Poole, 1988; Hirakow and Hiruma, 1981; Meier, 1980).

Formation of the DA in zebrafish has been studied by transmission electron microscopy (TEM) (Eriksson and Löfberg, 2000; Meier, 1980) and more recently by analysis of transgenic zebrafish embryos (Herbert et al., 2009; Jin et al., 2005; Lawson and Weinstein, 2002b). During vasculogenesis, angioblasts are attracted towards the midline by guidance cues thought to emanate from the endoderm