Developmental Biology 375 (2013) 182–192

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Evolution of Developmental Control Mechanisms

The evolutionary origins of chordate hematopoiesis and vertebrate endothelia

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ARTICLE INFO

Article history:
Received 21 August 2012
Received in revised form
16 November 2012
Accepted 19 November 2012
Available online 27 November 2012

Keywords:
Amphioxus
Hematopoiesis
Evo-devo
Endothelium
VEGFR-2/Flk-1
Scl/Tal-1

ABSTRACT

The vertebrate circulatory system is the most complex vascular system among those of metazoans, with key innovations including a multi-chambered heart and highly specialized blood cells. Invertebrate vessels, on the other hand, consist of hemal spaces between the basal laminae of epithelia. How the evolutionary transition from an invertebrate-type system to the complex vertebrate one occurred is, however, poorly understood. We investigate here the development of the cardiovascular system of the cephalochordate amphioxus *Branchiostoma lanceolatum* in order to gain insight into the origin of the vertebrate cardiovascular system. The cardiac markers *Hand*, *Csx* (*Nkx2-5*) and *Tbx4/5* reveal a broad cardiac-like domain in amphioxus; such a decentralized organization during development parallels that seen in the adult anatomy. Our data therefore support the hypothesis that amphioxus never possessed a proper heart, even transiently during development. We also define a putative hematopoietic domain, supported by the expression of the hematopoietic markers *Scl* and *Pdvegfr*. We show that this area is closed to the dorsal aorta anlages, partially linked to excretory tissues, and that its development is regulated by retinoic acid, thus recalling the aorta-gonads-mesonephros (AGM) area of vertebrates. This region probably produces *Pdvegfr*$^+$ hemal cells, with an important role in amphioxus vessel formation, since treatments with an inhibitor of PDGFR/VEGFR lead to a decrease of Laminin in the basal laminae of developing vessels. Our results point to a chordate origin of hematopoiesis in an AGM-like area from where hemal *Pdvegfr*$^+$ cells are produced. These *Pdvegfr*$^+$ cells probably resemble the ancestral chordate blood cells from which the vertebrate endothelium later originated.

Introduction

The vertebrate circulatory system, despite its high level of specialization and physiological relevance, nevertheless remains poorly understood, both in terms of its origin and its evolutionary transition from invertebrate hemal systems. Invertebrate hemal systems are usually composed of a network of cavities located between the basal laminae of epithelia (Ruppert and Carle, 1983). Frequently, these epithelia contain myofilaments and are contractile, contributing to the circulation of the hemal fluid. In these animals, the pumping organ is a specialized peristaltic vessel composed of myoepithelial cells. However, in vertebrates the endothelial cells delimit the vascular lumen and the heart is a multilayered and multi-chambered muscular organ. Although there exist important differences between the cellular elements involved in cardiovascular development of vertebrate and invertebrate phyla, a common basic gene network has been identified (Davidson and Erwin, 2006), suggesting that the extant circulatory systems and pumping organs of very diverged animals share a common evolutionary origin (Xavier-Neto et al., 2007). However, the evolutionary steps leading to the acquisition of complex vertebrate cardiovascular systems remain to be elucidated (Muñoz-Chápuli and Pérez-Pomares, 2010; Pérez-Pomares et al., 2009; Simões-Costa et al., 2005).

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0012-1606/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ydbio.2012.11.015

Hematopoiesis, the process that gives rise to the different blood cell lineages from hematopoietic stem cells (HSCs), generally takes place concomitantly with cardiovascular development. HSCs are responsible for maintenance and self-renewal of all blood cells in vertebrates (reviewed by Orkin and Zon 2008). During vertebrate ontogeny, hematopoiesis occurs first in the so-called blood islands (Galloway and Zon, 2003), situated in the extraembryonic tissues surrounding the yolk sac (or equivalent regions depending on the animal group), whereas in the embryo proper it occurs first in the aorta-gonads-mesonephros (AGM) region (Godin and Cumano, 2002; Robin et al., 2003). Members of the PDGFR/VEGFR (especially *VEGFR-2/Flk-1*) (Kattman et al., 2006) subfamily, as well as other tyrosine kinase receptors and the transcription factors SCL/TAL-1 and GATA1-3 (Gering et al., 1998; Pimanda et al., 2007) have a crucial function in hematopoiesis (reviewed by Cumano and Godin 2007). They are important elements of a gene regulatory network playing a key role in the determination of mouse HSCs in the yolk sac, in the AGM and in the fetal liver. Later in development, the endothelial lineage is marked by *VEGFR-2/Flk-1*, in contrast to the hematopoietic lineage. It is believed that both lineages originate from the same cellular progenitors, the hemangioblasts (Ema et al., 2003). Although the molecular mechanisms underlying hematopoiesis have been widely studied in vertebrate embryos and in embryonic stem cells, little is known about its evolutionary origin.

From an evolutionary point of view, three key issues are (i) the transition from the invertebrate to the vertebrate cardiovascular system, (ii) the evolutionary relationship between vertebrate and invertebrate hematopoiesis and (iii) the origin of vertebrate endothelium from invertebrate-type hemal cells. The cephalochordate amphioxus is placed in a key phylogenetic position to understand the origin of chordates (Bertrand and Escriva, 2011), as it represents the sister group of the tunicate–vertebrate clade (Delsuc et al., 2006). Amphioxus possesses a closed hemal system; the anatomical distribution of the main vessels and the direction of flow of hemal fluid (backwards dorsally and forwards ventrally) are reminiscent of those in the vertebrate embryo (Rähr, 1979). However, as has been widely described in the literature, adult amphioxus do not have a proper heart from a morphological point of view (Fig. 1), and the hemal fluid circulates by the contraction of several main vessels (depicted in Fig. 1) (Franz, 1927; Moller and Philpott, 1973; Rähr, 1981; Randall and Davie, 1980; Ruppert, 1997). However, if amphioxus develops a heart during development that is secondarily lost in the adult still remains to be investigated. As in other invertebrates, the contractile capacities of these vessels are due to myofilaments arranged basally in the coelomic epithelia (Moller and Philpott, 1973). Free hemal cells have been described within and lining the lumen of amphioxus vessels in some regions (Kučera et al., 2009; Rhodes et al., 1982). Kučera et al., (2009) described a possible role of these cells in the degradation of the extracellular matrix to open the vessel lumen, where Laminin is one of the main components. However, as in other invertebrates, a true endothelium is absent.

In order to better understand the transition from an invertebrate-type to a vertebrate hematopoietic and vascular system, we have analyzed a number of hematopoietic and cardiac markers in embryos of the European amphioxus *Branchiostoma lanceolatum*. Several cardiac markers have been previously studied in the Floridian amphioxus *Branchiostoma floridae*, although in some cases in a limited developmental window, such as *BMP2/4* (Panopoulou et al., 1998), *Csx* (*Nkx2.5/tinman*) and *Hand* (Holland et al., 2003; Onimaru et al., 2011), and partially *Tbx4/5* (Horton et al., 2008; Minguillon et al., 2009), leading to different conclusions. While Panopoulou et al., 1998 proposed the endostylar artery as a vertebrate heart homologue, Holland et al. (2003) proposed so for the subintestinal vessel. Furthermore, Onimaru et al. (2011) have suggested a separation of the amphioxus ventral mesoderm into an anterior pharyngeal domain and a posterior cardiac domain. Here, we study and extend the expression patterns of the cardiac markers *Csx* (*Nkx2-5/tinman*), *Tbx4/5* and *Hand* in *B. lanceolatum*, which define a broader cardiac area than previously reported including both pharyngeal and ventral trunk mesoderm. This suggests that all developing vessels in the pharynx (e.g., endostylar artery) and the trunk (e.g., subintestinal vessel), which are indeed contractile in the adult, represent the "cardiac" domain. On the other hand, the expression of three important hematopoietic markers (*Pdvegfr*, *Scl* and *Gata1/2/3*) suggests that during development, amphioxus embryos possess a hematopoietic domain in the anterior part of the body close to the two dorsal aortas, associated with the developing excretory system and regulated by retinoic acid (RA). This hitherto undescribed domain strongly resembles the vertebrate aorta-gonads-mesonephros area. Finally, using results from experiments in which we inhibit PDVEGFR, we discuss the putative function of

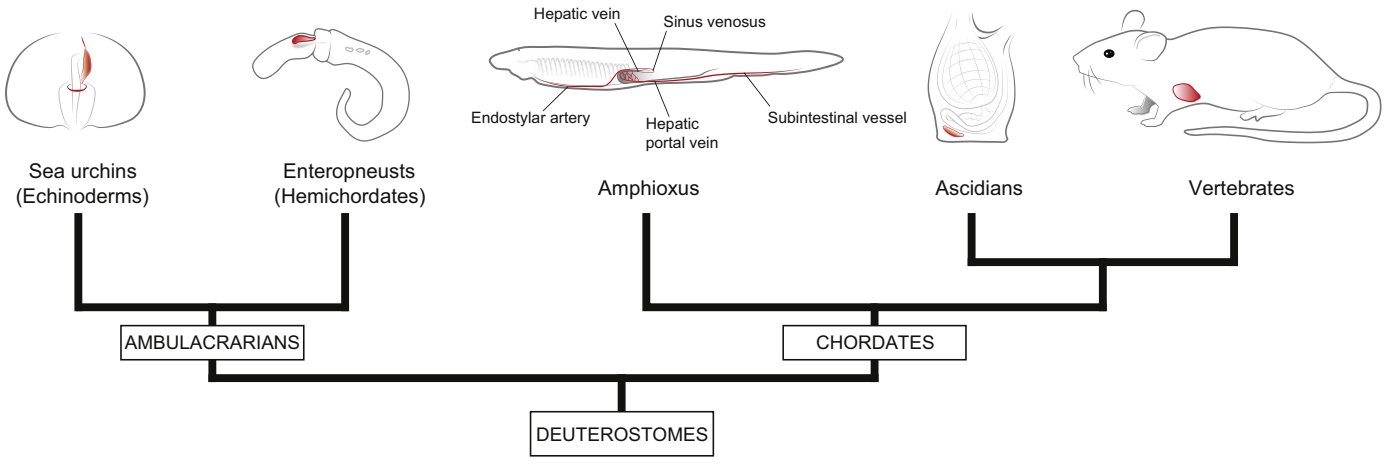

Fig. 1. Phylogenetic tree of deuterostomes depicting the heart and pumping organs. While echinoderms have a very specialized pumping organ, the axial organ, hemichordate enteropneusts have a heart–kidney complex on the rostral tip of the stomochord, in the prosome. Adult amphioxus are widely described as not possessing a proper centralized pumping organ or heart. Instead, several main vessels are contractile (labeled in the amphioxus scheme). Adult ascidians have a localized pumping vessel surrounded by a pericardium. Vertebrates possess complex chambered hearts, which represent an innovation of this group. The different pumping organs are colored in red, although the homology relationships between ambulacrarian and chordate pumping organs are still uncertain.