Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Vascular patterning: coordinated signals keep blood vessels on track

Stryder M Meadows[1] and Ondine Cleaver [2]

The vascular system is a complex, largely stereotyped network of interconnecting and branching vessels. How thousands of vessels form at precise locations is a key question regarding vascular morphogenesis. In order to achieve this defined architecture, the embryo integrates a multitude of attractive and repulsive cues to guide and shape the developing vasculature. This review summarizes recent studies investigating the interactions between endothelial cells and signals from surrounding tissues that pattern the initial blood vessel network.

Addresses
[1] Department of Cell and Molecular Biology, Tulane University, 2000 Percival Stern Hall, 6400 Freret Street, New Orleans, LA 70118, USA
[2] Department of Molecular Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA

Corresponding author: Meadows, Stryder M (smeadows@tulane.edu)

Current Opinion in Genetics & Development 2015, **32**:86–91

This review comes from a themed issue on **Developmental mechanisms, patterning and organogenesis**

Edited by **Deborah J Andrew** and **Deborah Yelon**

For a complete overview see the Issue and the Editorial

Available online 3rd March 2015

http://dx.doi.org/10.1016/j.gde.2015.02.002

Introduction

Endothelial cells (ECs) assemble to form an exquisitely sculpted vascular network in vertebrate embryos. How does this happen? The answer lies in the fine control of EC behavior by factors within the microenvironment. Depending on the molecular signal, ECs can be enticed toward sources of positive cues that enhance their migration, or redirected by repulsive signals that alter their directional motility. Through the combination of these instructions, a framework is formed in which blood vessels are corralled into defined locations where they assemble into functional vessels.

The vascular endothelial growth factor (VEGF) signaling pathway has taken center stage as a dominant player in endothelial biology, as it has been shown to be critical in regulating EC behavior and dynamics, including differentiation, survival, proliferation and migration [1]. VEGF has garnered attention as a powerful attractive cue influencing vessel patterning, as evidence through the years has demonstrated that VEGF, secreted from many different tissues, attracts and guides growing vessels as the vascular network is laid down and expanded [2]. However, it is unlikely that VEGF (and other attractive factors) alone can account for the precision inherent in vascular patterning since VEGF expression is also detected in domains/tissues devoid of blood vessels.

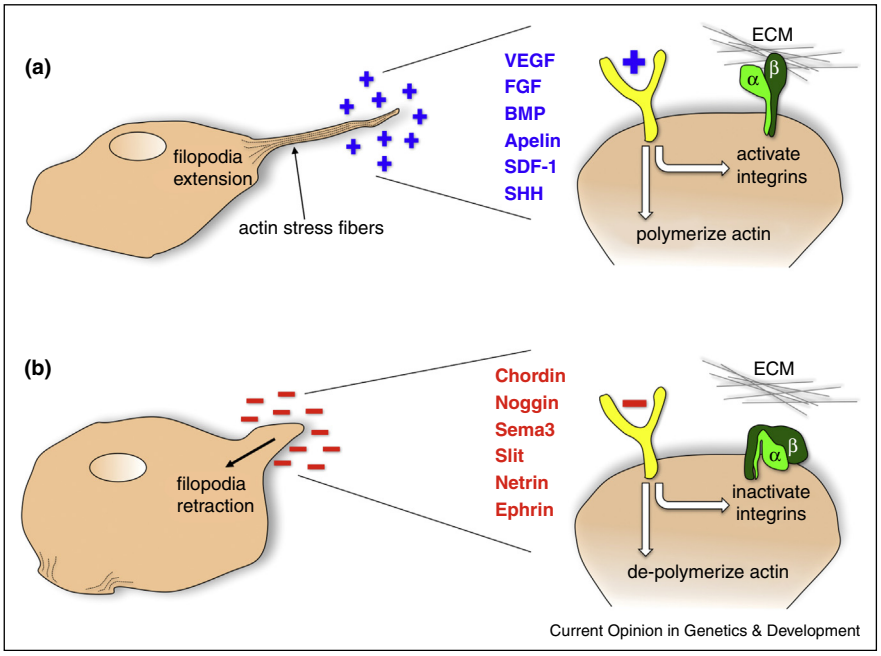
Indeed, progress in our understanding of the mechanisms of blood vessel patterning has come from a group of molecules termed neuronal guidance cues (NGCs) that were originally discovered to influence the guidance of axonal growth cones [3,4]. For the purpose of this review we will collectively identify these and other factors with inhibitory affects on EC behavior as repulsive guidance cues (RGCs). At the time, it was surprising to find many RGC receptors expressed by ECs, in addition to neurons. RGCs have since been shown to impact vessel growth and patterning in various model systems and in different vascular beds by countering the influence of VEGF on EC migration [4,5••,6]. At a cellular level, repulsive signals cause collapse of actin stress fibers and consequently cellular structures required for migration, such as filopodia and lamellipodia [7–10]. As a result, ECs are redirected in the opposite direction (Figure 1). This mechanism plays a major role in creating avascular zones that shape and pattern the vascular network by balancing the attractive properties of VEGF.

Given the increasing complexity of the vascular network during development and adulthood, many studies of the influence of guidance cues on early vessels have opted to examine the more simple vascular arrangements in the early embryo. This review will focus on these particular studies, and will discuss how attractive and repulsive factors secreted by adjacent tissues coordinate to create a microenvironment that affects EC dynamics and ultimately blood vessel patterning.

Notochord: a repulsive signaling center that shapes the first blood vessels

In avian and mammalian embryos, the first embryonic vessels to form are the paired dorsal aortae (DA). These large primitive vessels are formed via the process of vasculogenesis, whereby mesodermal derived precursor cells called angioblasts arise from the mesoderm *de novo*. The dorsal aortae are first evident as linear aggregates of angioblasts at distinct bilateral locations within

Figure 1



Regulation of endothelial cell motility by guidance cues. **(a)** In the presence of blood vessel promoting factors (blue) vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), bone morphogenetic protein (BMP), Apelin, stromal cell derived factor (SDF-1, also CXCL12) and sonic hedgehog (SHH), EC migration is directed toward the source of positive cues. In this scenario, actin polymerization leads to filopodial extension and integrin activation/association with the extra cellular matrix (ECM) to promote migration. **(b)** Conversely, repulsive guidance cues (red), such as the BMP antagonists Chordin and Noggin, Semaphorin 3 (Sema3), Slit, Netrin and Ephrin, cause actin de-polymerization and filopodia retraction, as well as integrin inactivation. In this situation, the EC is directed away from the source of inhibitory cues.

the embryo proper, around embryonic (E) day 8 of mice (Figure 2). As angioblasts differentiate into ECs, they coalesce into parallel tracts on either side of the centrally located notochord and subsequently transform into patent tubes. As development proceeds, the DA shift medially, fusing into a single midline aorta and taking a position immediately ventral to the notochord. The process by which two distinct vessels form and remain separate before ultimately creating a single structure represents a unique, yet simple platform for understanding the dynamics of vascular patterning.

The Mikawa group first demonstrated in avian embryos that the notochord, which lies at the embryonic midline, creates an avascular zone separating and subsequently patterning the DA before their fusion [11••]. Removal of the notochord in chicken embryos allowed unrestrained EC migration and intermingling of sprouts between adjacent vessels, disrupting DA formation, while transplantation of the notochord into highly vascularized regions of the embryo created avascular areas. These studies further identified notochord-expressed bone morphogenetic protein (BMP) antagonists, Chordin and Noggin, as repulsive signals capable of recreating avascular domains *in vivo*. Together, this work pinpointed embryonic non-vascular tissues as important sources of cues directing the formation of the vasculature, signaling where ECs could and could not reside.

More recently, we showed that the mammalian notochord plays a similar role in patterning the DA. Foxh1 and FoxA2 null embryos that lack a notochord exhibit DA patterning defects similar to those seen in chicks upon notochord extirpation [12••]. We found that like the avian notochord, the mammalian notochord expressed numerous molecules known to repel ECs. In addition to Chordin and Noggin, the mouse notochord expresses the RGCs Slit2, Netrin1 and Semaphorin 3E (Sema3E), while their cognate receptors are present on ECs of the DA. Loss of Sema3E resulted in loss of normal aortic patterning and marked narrowing of the midline avascular zone (Figure 2). The co-expression of repulsive cues in the notochord of the early embryo likely represents a broader theme, where multiple fields of cues overlap and coordinate to direct blood vessel patterning in a robust and reproducible manner (Figure 2).

In addition, work on dorsal aorta formation underscored how cues act on angioblast migration at specific times and places. Together, the findings discussed above demonstrate that the notochord secretes multiple repulsive cues