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The same but different: signaling pathways in control of endothelial cell migration

Sana S Hasan and Arndt F Siekmann

The coordinated migration of endothelial cells (ECs) plays a pivotal role not only in the assembly of the embryonic vasculature, but also during various physiological and pathological processes, such as tissue regeneration and wound healing. Recent reports studying EC migration in distinct vascular beds have revealed common principles, but also surprising differences, in the molecules ECs use to ensure proper migratory behaviors. In addition to genetic cues, hemodynamic forces in perfused blood vessels also affect EC migration, thereby contributing to blood vessel remodeling. In this review, we will discuss the distinct molecules guiding EC migration in different tissues and highlight their modes of action.

Address
Max Planck Institute for Molecular Biomedicine, Roentgenstr. 20, 48149, Muenster, Germany

Corresponding author: Siekmann, Arndt F (arndt.siekmann@mpi-muenster.mpg.de)

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Introduction

Blood vessels are one of the first systems to develop in early vertebrate embryos [1]. ECs typically form a cobbled framework lining the inner part of all blood vessels. During blood vessel development, ECs undergo numerous morphological and behavioral changes. The process involves cell division, establishment of polarity, cell intercalation and rearrangement [2]. Since ECs need to invade avascular tissues during blood vessel formation, a tight control of their migratory properties is necessary. Work over the recent years has revealed a surprising context-dependent variability in the molecules ECs use for controlling their migratory behaviors. ECs may exhibit single-cell migration during vasculogenesis, or collective migration during sprouting angiogenesis [3]. Collective cell migration poses additional constraints on the migrating cells, such as maintaining junctional integrity with neighboring cells and organization of multicellular polarity [4]. At later stages of blood vessel formation, EC motility is important for the extensive cellular rearrangements that occur during lumen formation and blood vessel pruning. This review discusses the recent advances in our understanding of EC migration at different developmental time points and in distinct organs, with an emphasis on the molecules guiding EC migration.

Endothelial cell migration during vasculogenesis

The establishment of the primary vascular network during embryonic development occurs via vasculogenesis. It is defined as the *de novo* formation of blood vessels, which involves the assembly of individual angioblasts (endothelial progenitors) into vascular cords [5,6]. In zebrafish and Xenopus embryos, angioblasts arise in the lateral plate mesoderm and migrate to the midline, where they form the first artery and vein [7,8]. This migration is affected in embryos lacking medially located structures, such as the notochord [9–11]. Initial studies in Xenopus embryos implicated Vascular Endothelial Growth Factor (VEGF) as an important angioblast chemoattractant [7]. Subsequent studies in zebrafish identified endothelial cell-specific chemotaxis receptor (ECSCR) to promote angioblast migration by enhancing the sensitivity of VEGF receptor 2 (VEGFR2) to surrounding VEGF [12]. However, knocking down either VEGF ligand expression [13,14] or its downstream signaling mediator plcg1 [13] did not alter initial angioblast migration. Therefore, the influence of VEGF signaling on angioblast migration in early embryos remains controversial.

Another important regulator for the formation of the early embryonic vasculature is hedgehog signaling. Both in zebrafish and mouse, the dorsal aorta (DA) forms before the cardinal vein (CV), by two waves of angioblast migration [15–19]. In hedgehog-deficient embryos, there is an absence of the first wave of angioblast migration [20]. Consequently, all angioblasts contribute to the second migratory wave and therefore to CV formation. Surprisingly, despite an additional absence of VEGF expression in hedgehog-deficient embryos [21], angioblasts still arrive at the midline position, further suggesting that both signaling pathways, in spite of their importance for angioblast proliferation and arterial-venous differentiation, do not influence initial angioblast migration.

Recent work by Helker *et al.* now identified Apelin receptor signaling as an important pathway controlling

angioblast migration to the midline. Elegant transplantation experiments revealed that apelin receptors are required cell autonomously for proper angioblast migration, further illustrating that angioblasts migrate as single cells [22].

Endothelial cell migration during angiogenesis

Angiogenesis is the formation of new blood vessels from pre-existing ones and is the major source of new blood vessels after the completion of vasculogenesis [23]. ECs in patent blood vessels normally have a very limited migratory and proliferative activity. However, in response to pro-angiogenic cues, ECs become activated and start to sprout [2]. Two important genetic pathways influencing these processes are the VEGF and Notch signaling pathways. Under hypoxic conditions, local tissues secrete VEGFA, which binds to VEGFR2 on ECs, thereby initiating cell proliferation and migration. In addition, VEGF signaling induces the expression of the Notch ligand delta-like 4 (Dll4). Dll4 binds to Notch receptors on adjacent cells, activating Notch signaling, which suppresses angiogenic sprouting. Ultimately, this leads to the specification of leading tip and trailing stalk cells [24]. Tip cells are characterized by a highly migratory and polarized phenotype, extensively extending actin-rich filopodia [25,26]. Studies in several cell types have implicated filopodia as important structures during the sensing of extracellular cues and for guided cell migration [27], including ECs in angiogenic blood vessel sprouts [25].

Which factors control filopodia formation in ECs? Serum response factor (SRF) is an evolutionary conserved transcription factor known to be important for filopodia formation in *Drosophila melanogaster* tracheal cells [28]. Inducible endothelial specific knockout of SRF resulted in organizational defects in actin polymerization and contractility, which translated into defects in filopodia formation and severe defects in retinal vasculature development in mouse [29]. Importantly, SRF function was specifically required for cell motility in leading tip cells but not in stalk cells, suggesting that different modules control the migratory properties of these two cell types [29]. In addition to extending protrusions in the direction of migration, ECs also need to retract their plasma membranes at the rear end. Vitorino *et al.* elucidated the role of MAP4K4 during this process [30••]. The authors found that MAP4K4 kinase was necessary to phosphorylate moesin during EC migration. Phosphorylated moesin in turn replaced talin from b1-integrin at focal adhesion sites, thereby allowing for membrane retraction (Figure 1).

A recent study by De Bock *et al.* showed that differences in metabolism could also control EC migration [31••]. The authors demonstrated that ECs relied on glycolytic ATP production for proper filopodia and lamellipodia formation during EC migration. They identified Phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFKFB 3), a stimulator of glycolysis, to co-localize with F-actin in motile protrusions, which resulted in compartmentalized ATP generation. This in turn was instrumental for proper actin remodeling. Accordingly, silencing of PFKFB 3 in ECs resulted in impaired EC migration [31••]. Together, these studies indicate that distinct signaling modules exist in ECs that are necessary to coordinate the intricate cell migratory behaviors during angiogenic sprouting.

EC migration in different vascular beds

Of note, two recent studies in zebrafish embryos have investigated the influence of filopodia on angiogenesis and EC migration in zebrafish intersegmental vessels (ISVs) [32••] and the posteriorly located caudal vein plexus (CVP) [33••], respectively (Figure 2). Previous studies showed that both vascular beds rely on different signaling pathways for their proper formation. While ISVs heavily rely on VEGF signaling [14], CVP formation can occur in the absence of VEGF signaling, but depends on bone morphogenetic protein (BMP) signaling [34]. During ISV formation, filopodia are dispensable for tip cell guidance and only mildly affect EC migration speed [32••]. In this setting, ECs generated lamellipodia that could drive migration. By contrast, the analysis of CVP formation showed that filopodia are indispensable for proper venous sprouting. Wakayama *et al.* identified formin-like 3 (fmnl-3), an actin regulatory protein, which promotes extension of actin filaments to critically influence filopodia formation and EC migration in venous cells [33••]. The authors demonstrated that BMP signaling regulated Arhgef9b, a guanine exchange factor, which in turn activated Cdc-42. Activated Cdc-42 increased fmnl-3 activity by binding to the latter's N-terminus. Of interest, during ISV formation, fmnl-3 activity is instead required for proper lumen formation and maintenance [35].

Differences in downstream signaling between VEGF and BMP might explain the contrasting requirements for filopodia during ISVs and CVP formation. VEGF signaling can activate both Cdc-42 and Rac1, required for filopodia and lamellipodia formation respectively (Figure 3). Therefore, in the absence of filopodia, ECs within ISVs can still migrate via lamellipodia protrusions. CVP formation requires BMP signaling, which is known to activate Cdc-42 but not Rac-1 [33••]. Therefore, EC migration during CVP formation might fail in the absence of filopodia due to inability of these cells to form lamellipodia. Further studies are needed to confirm this hypothesis. Together, these reports suggest that ECs show a context-dependent requirement for proper filopodia formation in controlling directed cell migration.

Angiogenic sprouting and migration in complex vascular beds requires additional cues for proper guidance. Studies in zebrafish embryos have shown that the sequential activation of VEGF and chemokine signaling via Cxcr4