Vascular morphogenesis: an integrin and fibronectin highway

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A new study shows that endothelial cells use synaptic-like machinery to control polarized secretion and deposition of newly synthesised fibronectin. This process is coupled to active integrin recycling to the same locations and is fundamental for vascular development in zebrafish.

The formation of new blood vessels and subsequent maturation into a functional tubular network that supports blood circulation are the defining events for embryonic development. Unsurprisingly, the elements that control vascular morphogenesis are also implicated in multiple life-threatening human diseases (e.g. ischemic cardiovascular disease and neoplastic development) and as such vascular biology has become an intensely studied research topic. Vascular development is highly dependent on the continued and extensive crosstalk between endothelial cells that line the vessel wall and the underlying basement membrane, a specialized proteinaceous extracellular matrix (ECM). In particular, endothelial cells secrete fibronectin, a key ECM protein, as a soluble dimer that is then reorganized into a fibrillar network outside of the cell. In this bioactive fibrillar form, fibronectin provides important mechanical and chemical cues necessary for endowing endothelial cells with a sense of polarity during vascular tubulogenesis [1-3]. Endothelial cell interactions with the ECM are predominately mediated by integrins, a family of transmembrane heterodimeric adhesion proteins consisting of α and β subunits. Integrins bind directly to ECM ligands, trigger important signaling pathways and provide a physical anchor between the cell cytoskeleton and the ECM. The fundamental requirement for fibronectin and its receptor, fibronectin-binding integrin α5β1, in cardiovascular development is apparent from genetic studies where the ablation of fibronectin or the integrin α5 subunit or a single mutation in the integrin-binding RGD sequence in fibronectin lead to embryonic lethal vascular defects in mice [4,5]. In a new study, Mana et al. outline a novel endo-exocytic circuit in endothelial cells that supports fibronectin fibrillogenesis [6]. They find that endocytosis and removal of “old” proteolytically cleaved fibronectin is coupled to the polarized exocytosis of newly synthesized fibronectin at the basolateral membrane. Moreover, they show that this process

requires intact interactions between endothelial cells and the ECM and is critical for the formation of a functioning vascular network in zebrafish embryos [6].

It is important to note that integrin-ECM adhesions are not static unchangeable structures and that dynamic remodeling of the ECM by endothelial cells is critical for vascular morphogenesis [7,8]. Fibronectin contains multiple sites for other matrix proteins and as such its turnover and reorganization can have a profound effect on overall ECM structure and stability [8,9]. Fibronectin remodeling is a balance between removal of "old" fibronectin fibrils and the deposition of newly synthesized fibronectin dimers, followed by fibrillogenesis. Importantly, in endothelial cells, and in contrast to fibroblasts, fibronectin secretion has to occur in a polarized manner at the basolateral membrane to maintain cell polarity. Integrins are also turned over in a process that is tightly regulated and typically referred to as receptor trafficking. Integrin traffic involves receptor internalization at the plasma membrane, trafficking through different endocytic vesicles and recycling back to the plasma membrane to form new adhesions [10,11]. A role for integrins in fibronectin fibrillogenesis has been previously documented, where integrin binding to fibronectin dimers promotes integrin clustering and therefore fibronectin-fibronectin interaction and fibrillar assembly [12]. Integrins have also been implicated in matrix metalloproteinase-cleaved fibronectin endocytosis leading to lysosomal targeting [13]. Interestingly, interfering with integrin traffic, by blocking receptor internalization, inhibits fibronectin fibrillogenesis [14]. Whether this is due to accumulation of excess cleaved fibronectin in the microenvironment or aberrant signaling from integrins is not clear. Regardless, it seems that the presence of active integrins on the cell surface and direct binding to fibronectin is not enough to support fibril formation. Instead, integrin traffic may be an underappreciated player controlling new fibronectin fibrillogenesis.

Mana et al., addressed this unexplored area by taking lessons from neurons, where controlled endo-exocytic traffic and polarized docking of neurotransmitter-containing vesicles to specific membrane compartments are critical for cellular function. Liprin α1, also known as protein tyrosine receptor type f polypeptide (PTPRF) interacting protein α1 (PPFIA1) is a ubiquitously expressed adaptor protein, originally identified as a regulator of synapse formation and function [15]. Additionally, PPFIA1 couples endocytosis and exocytosis in presynaptic nerve termini [15] and was recently identified as a component of integrin-containing adhesions [16]. Mana et al., show that PPFIA1 localizes to fibrillar adhesions

(α5β1-integrin-positive cell-ECM contacts and sites of fibronectin fibril generation) at the basolateral membrane in endothelial cells, binds directly to the integrin β1-tail and in line with its role in synapses regulates recycling of active endocytosed integrins. The absence of PPFIA1 resulted in the accumulation of α5β1 integrin in post-Golgi carriers (PGCs), suggesting that PPFIA1 affects integrin recycling back to the plasma membrane. Importantly, newly synthesized cellular fibronectin also accumulated in the same PGCs as α5β1 integrin, and fibronectin secretion was impaired specifically at the basolateral membrane in the absence of PPFIA1 [6]. Surprisingly, simultaneous inhibition of integrin endocytosis (through Rab21 silencing [17]), and integrin recycling back to the plasma membrane (through PPFIA1 silencing) appeared to rescue the defects in fibronectin fibrillogenesis observed in the absence of either adaptor [6]. Thus, deposition of fibronectin fibrils appears to be functionally balanced between endocytosis of old fibrils and exocytosis of new fibronectin coordinated by α5β1 integrin, Rab21 and PPFIA1.

Mana et al., further define the molecular pathway that couples integrin recycling to polarized fibronectin secretion and find that regulators of PGC biogenesis, and PTPRF, which together with PPFIA1, regulates synapse formation, cooperate to compartmentalize fibronectin fibrillogenesis at the basolateral membrane (summarized in Figure. 1). They show that this pathway is biologically relevant for vascular morphogenesis in vitro and in vivo. Specifically, in the absence of PPFIA1, endothelial cells failed to form cell-cell contacts, to lay down a fibronectin meshwork or to form capillary structures in capillary formation assays. In zebrafish embryos, knockdown of PPFIA1 protein expression triggered cardiovascular defects including malformation and irregularities in vessel shape, an enlarged heart chamber and reduced blood flow in 35 % of the embryos [6].

Altogether, Mana et al., describe a novel exocytosis mechanism in endothelial cells that shares components with the neurotransmitter docking system in nerve termini. This pathway mediates fibronectin deposition at cell-ECM adhesions in an α5β1-integrin-dependent manner (Figure. 1). Importantly, this study demonstrates for the first time that the dynamic traffic of integrins in cells is coupled to secretion of new ECM components and is not limited to degradation and turnover of existing ECM.

However, cell adhesion to fibronectin is not solely performed by α5β1 integrin. Integrin αvβ3 is a fibronectin and vitronectin specific receptor and is expressed by endothelial cells [4].

Although genetic knockouts of this receptor are not embryonic lethal, αvβ3 is upregulated during angiogenesis [4]. Integrin αvβ3 has also been shown to be able to promote fibronectin fibrillogenesis through a different mechanism to α5β1 integrin [5], although the relevance of this in vivo remains to be investigated. Additionally, there is a great degree of interplay between α5β1 and αvβ3 integrin recycling pathways, which in cancer cells determines the mode of 3-dimensional cell migration in a fibronectin-dependent manner [11]. Whether PPFIA1 and the exocytosis machinery identified by Mana et al., also regulates αvβ3 integrin traffic is not known. Another interesting avenue to investigate is whether this pathway is employed in developed tissues under specific pathophysiological conditions that are associated with increased remodeling and/or miss-targeted fibronectin deposition. Although, Mana et al., suggest that the PPFIA1 exocytosis machinery does not affect apical fibronectin secretion, this remains to be fully determined under disease conditions where loss of polarity signals or abnormally increased integrin activity may disturb the direction of exocytosis. In addition, the role of this newly identified fibronectin highway in vascular homeostasis remains to be studied.

This study implicated a specific role for active α5β1 integrin, endocytosed in a Rab21-dependent manner, in fibronectin deposition. The continued traffic of Rab21-internalized integrins has, in turn, been shown to require a switch between endosomal Rab21 and p120RasGAP binding to the β1-integrin tail to release a prohibitory signal that would otherwise impair integrin recycling to other compartments [18]. Interestingly, ablation of p120RasGAP leads to embryonic lethality due to defects in vascular development [19]. Whether this is linked to the requirement of α5β1 recycling for fibronectin remodeling, identified by Mana et al., or solely to the role of p120RasGAP in regulating Ras signaling remains to be investigated.

Integrins have recently been shown to act as non-canonical receptors for the angiopoietin-2 growth factor, an important regulator of vascular homeostasis [20]. Angiopoietin-2 binding to α5β1 integrin was shown to activate the integrin receptor leading to destabilized endothelial cell-cell junctions and altered fibronectin fibrillogenesis [20]. Given the changes in fibronectin fibril formation, it would be interesting to reveal if angiopoietin-2-mediated integrin activation regulates the endo-exocytic pathway outlined by Mana et al. Indeed, vascular development and morphogenesis is a complex and tightly regulated process and mounting evidence highlights the fundamental requirement for the coordinated activity of

growth factor and integrin-ECM signalling pathways in establishing and maintaining a normal functioning vascular network.

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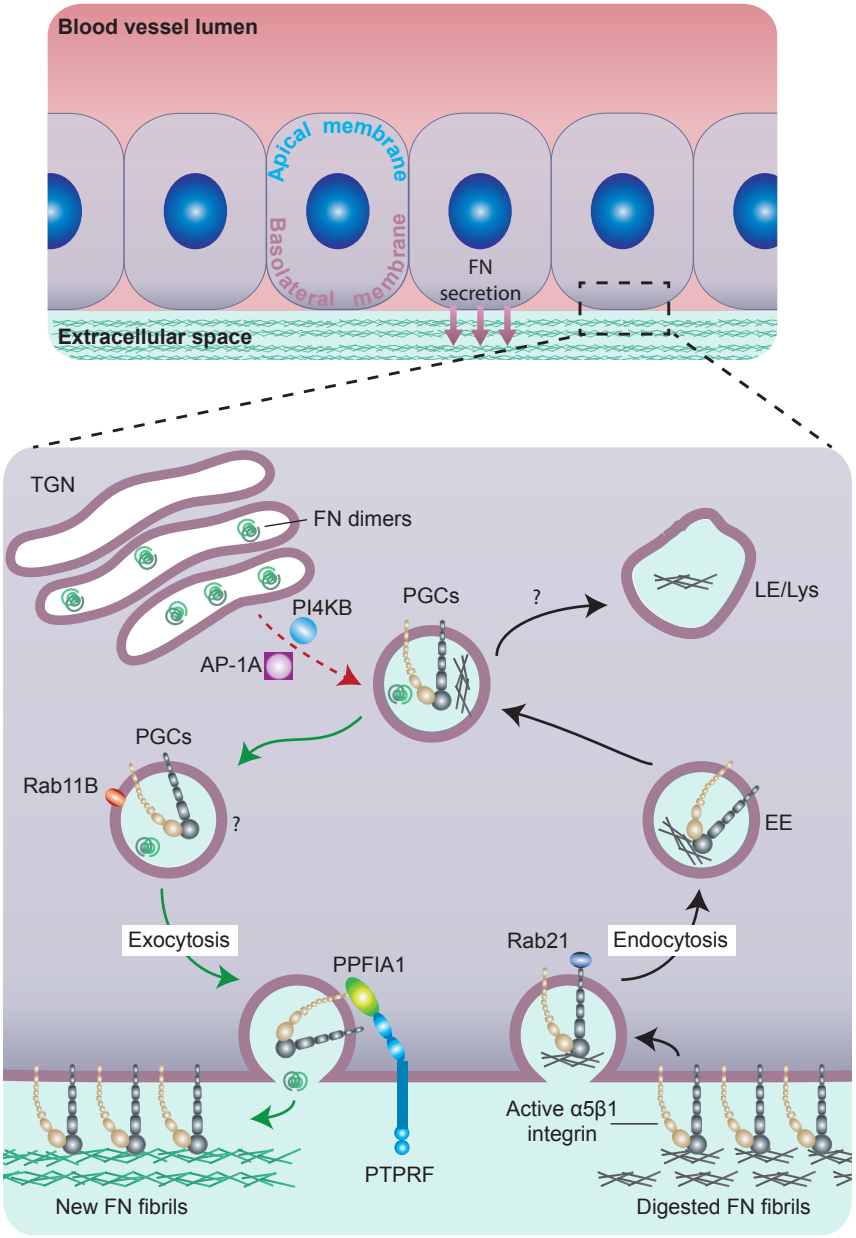
Figure 1. Summary of the endothelial fibronectin exocytosis pathway outlined by Mana et al. In endothelial cells, matrix metalloproteinase-cleaved fibronectin is internalised with active α5β1integrin in a Rab21-dependent manner and travels along the endo-exocytic route (black arrows). In PGCs these "old" fibronectin fragments are replaced with freshly synthesised fibronectin dimers. While the old fibronectin is likely targeted to lysosomes for degradation, the active α5β1 integrin and the new fibronectin dimers proceed down the exocytosis route towards the basolateral membrane in a Rab11B-dependent manner (green arrows). However, it is not known whether α5β1 integrin binds directly to the fibronectin dimer or simply acts as a positional cue to drive new fibronectin towards the basolateral membrane. PPFIA1 binds directly to α5-integrin and in complex with PTPRF localizes in close proximity to integrin adhesions, where it is thought to favour the docking of the integrin-fibronectin-containing PGCs.

AP-1A: endothelial cell-expressed clathrin adaptor protein complex-1A, involved in protein sorting from the TGN to PGCs; EE: early endosomes; FN: fibronectin; LE/Lys: late endosomes/lysosomes; PGCs: post-Golgi carriers. PI4KB: phosphatidylinositol 4-kinase, catalytic, beta, involve in protein sorting from TGN to PGCs; PPFIA1: protein tyrosine receptor type f polypeptide (PTPRF)-interacting protein α1; Rab: Ras-related proteins in brain. Rabs are GTPase signaling molecules and critical regulators of receptor trafficking pathways, where different family members play unique roles and decorate different vesicular compartments; TGN: trans-Golgi network.


Blood vessel lumen
Apical membrane
Basolateral membrane
FN secretion
Extracellular space
TGN
FN dimers
PI4KB
AP-1A
PGCs
?
LE/Lys
PGCs
Rab11B
?
EE
Exocytosis
Endocytosis
Rab21
PPFIA1
Active α5β1 integrin
PTPRF
New FN fibrils
Digested FN fibrils