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DISPATCH

Angiogenesis: The Importance of RHOJ-mediated Trafficking of Active Integrins

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In endothelial cells, endo-exocytic traffic of active α5β1 integrins and polarized fibronectin secretion allow vascular morphogenesis. A new study unveils how the endothelial small GTPase RHOJ, by repressing active α5β1 integrin traffic, controls fibronectin polymerization and *in vivo* angiogenesis.

In vertebrates, the formation of functional blood vessel trees ensures the effective delivery of oxygen and nutrients to tissues and organs along with the removal of waste products. In the developing embryo, endothelial cell (EC) progenitors first self-assemble by vasculogenesis into primitive vessels, which are then reshaped by sprouting angiogenesis (and fusion) into hierarchically-organized and arborized vascular networks, finally enabling the optimal circulation of blood throughout the body [1]. Similarly, cancer growth also relies on newly formed angiogenic vasculature. However, blood vessels of most solid tumors display abnormal structure and function, suboptimal perfusion, and hypoxia that instigates metastatic dissemination [2].

Dynamic adhesive cell interactions to surrounding extracellular matrix (ECM) protein polymers are crucial for assembly, remodeling, and stabilization of endothelial tubes [3]. Among ECM components, fibronectin (FN) supports blood vessel formation and angiogenic rehandling, while laminins impair vascular morphogenesis and maintain the stability of mature arteries, capillaries, and veins [3]. EC adhesion to the ECM is mediated by integrins, which are a family 24 heterodimeric transmembrane receptors, each arising from the combination of different α and β subunits [4]. Both FN

and its major endothelial receptor α5β1 integrin, in cooperation with αvβ3 integrin, are fundamental for physiological vascular morphogenesis *in vivo* [5].

Integrins can display either a bent/closed (inactive) or an extended/open (active) conformation that respectively binds ECM ligands with low and high affinity [6]. On the cell surface, inactive integrins are homogeneously distributed, whereas active integrins are highly enriched in ECM adhesion sites. As a rule, morphogenetic events rely on the modulation of cell adhesion to the ECM in space and time. This fine control depends not only on the regulation of integrin allosteric conformation [6], but also endosomal traffic to and from the plasma membrane of both inactive and active integrin conformers *via* a complex, and only partially characterized, network of functionally distinct pools of cytosolic vesicles [4, 7]. In this issue of *Current Biology* Sundararaman *et al.* [8] unveil how the endothelial small GTPase RHOJ, which is known to control vascular morphogenesis [9, 10], acts by selectively regulating the traffic of active, but not inactive α5β1 integrin, thus finely tuning the assembly of FN into polymeric fibrils both in cultured ECs and *in vivo*, thus allowing physiological angiogenesis to occur (Summarized in Figure 1).

The turnover of fibrillar FN networks is key to both physiologic and pathologic angiogenesis. In ECs, upon matrix metalloprotease-dependent cleavage of FN fibrils, FN fragment-bound active α5β1 integrins are internalized in a RAB21-dependent manner in early endosomes and trafficked to post-Golgi carrier (PGC) vesicles that bud from the *trans*-Golgi network (TGN) and contain freshly synthesized FN [11]. Once in PGCs, labelled by TGN protein 46 kD (TGN46), active α5β1 integrins detach from FN fragments and, together with new FN dimers, are trafficked along a RAB11B-dependent route to the basolateral side of the endothelial plasma membrane [11]. Here, PGCs exocytose freshly synthesized FN and recycled active α5β1 integrins close to existing fibrillar adhesions under the control of the protein tyrosine phosphatase receptor type f polypeptide (PTPRF)/PTPRF interacting protein a1 (PPFIA1, aka liprin-α1) complex [11] (Figure 1). Recent proteomic studies established that, in addition to FN, PPFIA1/liprin-α1 also drives the basolateral secretion of the basement membrane organizing protein

dystroglycan-1 [12]. Therefore, the PPFIA1/liprin-α1 adaptor may control the polarized exocytosis of ECM proteins in ECs of both angiogenic and quiescent blood vessels [3]. In this context, a major issue is represented by the identification of the molecular mechanisms in charge of directing internalized active α5β1 integrins towards a recycling or degradative fate in PGCs or late endosomes/lysosomes, respectively.

Prototypical RHO small GTPases, such as RHOA, RAC1 and CDC42 are well-known for their crucial function in the control of actin polymerization, cell shape and cell motility. However, archetypal RHO GTPases along with new family members, appeared during vertebrate evolution (*e.g.* RHOC and RHOG), their downstream effectors, and the actin cytoskeleton have also been involved in several steps of transmembrane receptor traffic [13], ranging from internalization to endosomal sorting towards either plasma membrane or TGN [4, 13]. Furthermore, CDC42 was recently shown to regulate bidirectional transport within the Golgi apparatus [14] and anterograde transport from the TGN to the plasma membrane [15].

Sundararaman *et al.* [8] provide novel evidence about the role that the CDC42-related RHO family member RHOJ plays in controlling active α5β1 integrins internalization and post-endocytic traffic in ECs, where this small GTPase localizes in early endosomes (EEs), late endosomes/lysosomes (LEs/LYs), but not PGCs. α5β1 integrin is the main cargo of RHOJ containing vesicles. In ECs, the lack of RHOJ increases, while the overexpression of constitutively active RHOJ decreases, the amount of active α5β1 integrin that localizes in elongated fibrillar adhesions. The Authors also reveal how, in cultured ECs, RHOJ silencing favors the sorting of endocytosed active, but not inactive α5β1 integrins towards PGCs, while reducing their accumulation in the LE/LY compartment. Intriguingly, the absence of RHOJ also increases the quantity of active, but not inactive α5β1 integrins that progressively accumulates in endosomes, particularly at later time points after internalization. On the contrary and differently from PTPRF and PPFIA1 [7, 11], RHOJ does not exert any effect on the recycling to the cell surface of

endocytosed active or inactive α5β1 integrin. These data may suggest that in ECs RHOJ inhibits active α5β1 integrin internalization from fibrillar adhesions. However, the significantly higher late accumulation of endocytosed active α5β1 integrins in PGCs rather than LEs/LYs in ECs devoid of RHOJ may instead imply that RHOJ may principally act by promoting lysosomal degradation of a sizeable fraction of active α5β1 integrins. Further investigations are required to clarify this aspect.

In agreement with their conclusions on active α5β1 integrins, Sundararaman *et al.* [8] show that RHOJ silencing enhances the ability of ECs to polymerize both endogenous and exogenous soluble FN into an insoluble matrix, while active RHOJ overexpression results in the opposite effect. Since endogenous FN secretion is not affected by either the lack or the constitutive activation of RHOJ, it is conceivable that the effect of RHOJ on FN polymerization in ECs is not direct, rather deriving from the regulation of active α5β1 integrin levels. Of note, the Authors also report that, when compared to RHOJ, silencing its close relative CDC42 or overexpressing a constitutively active isoform of it result in fully complementary effects on FN polymerization, suggesting that CDC42 and RHOJ are two closely related small GTPases that may reciprocally inhibit each other to control active α5β1 integrin levels and FN fibrillogenesis. Sundararaman *et al.* [8] identify p21 (RAC1) activated kinase 3 (PAK3) as a shared effector protein for whose binding CDC42 may compete with RHOJ. PAK3 interacts with higher affinity with CDC42 than with RHOJ. Indeed, similarly to CDC42, silencing or overexpression of PAK3 in ECs respectively impairs or increases the polymerization of endogenous FN without affecting its secretion. Furthermore, activated RHOJ suppresses the promotion of FN fibrillogenesis elicited by PAK3 overexpression. However, ECs lacking PAK3 do not display alterations of active α5β1 integrin endocytosis. Therefore, shared effector proteins other than PAK3 are likely involved in the antagonistic control of active α5β1 integrin traffic and FN fibrillogenesis by RHOJ and CDC42 in ECs. In this regard, in addition to PAK1 [8], formin-like protein 3 (FMNL3), which mediates the CDC42-driven anterograde

traffic from TGN to plasma membrane [15] and participates in the control of RHOJ on EC polarity [16], is an attractive candidate.

Finally, Sundararaman *et al.* [8] also reveal how FN deposition markedly increases around retinal blood vessels of *RHOJ* knock-out mice, which are significantly delayed in radial growth and characterized by a marked increase of empty ECM sleeves [9]. Altogether, these data bolster the *in vivo* relevance of the RHOJ-dependent control of active α5β1 integrin traffic and FN fibrillogenesis in ECs. During physiological blood vessel formation, the action of angiogenic stimulators, such as vascular endothelial growth factor (VEGF), is balanced by angiogenic inhibitors, such as semaphorins [2]. This equilibrium is lost in pathological cancer angiogenesis [2], *e.g.* due to hypersecretion of VEGF or lack of semaphorins [17, 18]. The notion that VEGF [19] and semaphorins [20] oppositely modulate CDC42 and RHOJ activation in ECs further emphasizes the implications of the study of Sundararaman *et al.* [8].

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Declaration of Interests

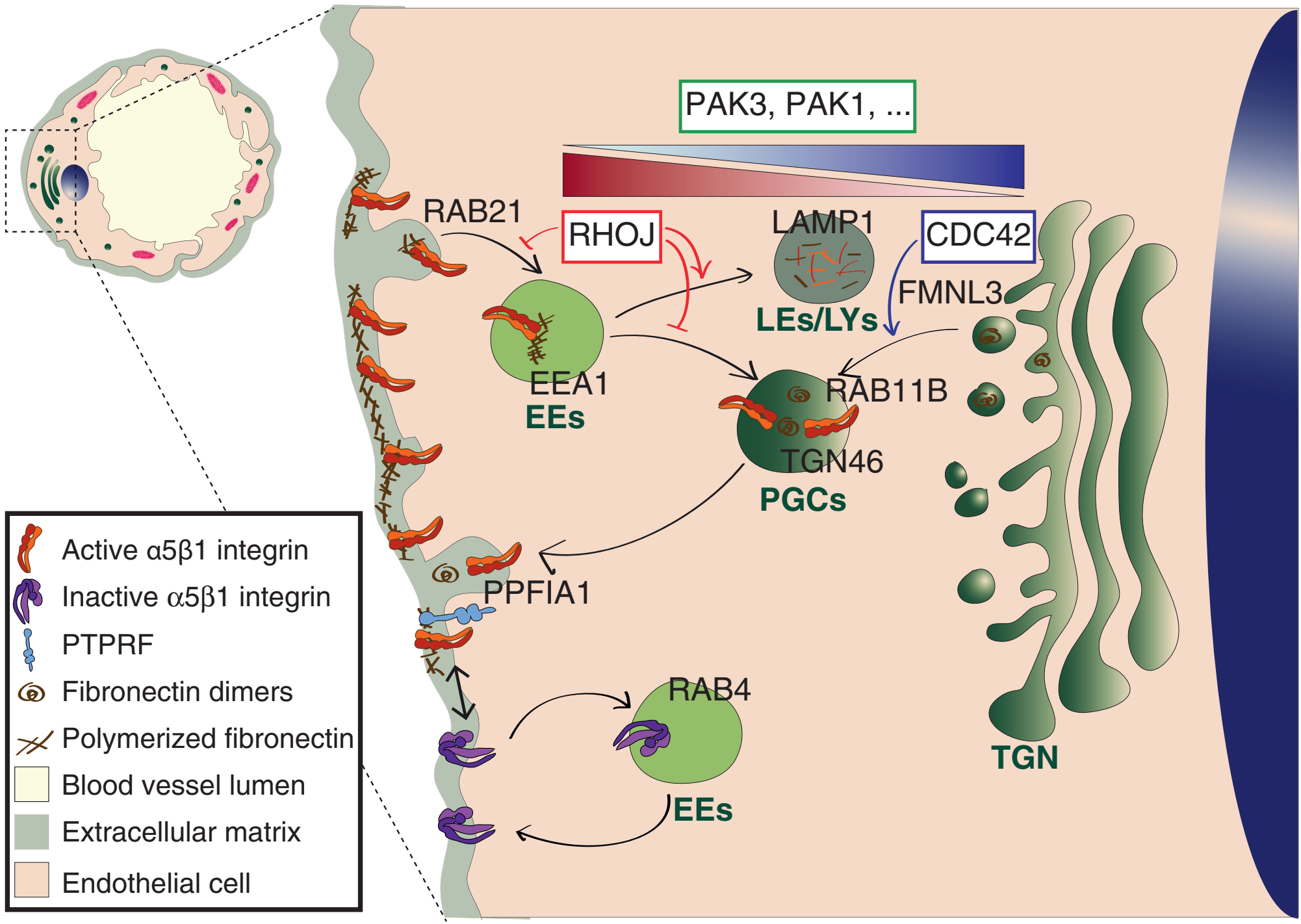
G.S. is co-inventor of a patent application on “Non-natural semaphorins 3 and their medical use” (international application no. PCT/EP2016/053750), co-cofounder and shareholder of SeaGull Therapeutics SAS that is developing mutant semaphorins for clinical applications.

D.V. declares that she has no competing interests.

Figures

Figure 1. Reciprocal inhibition of closely-related small GTPases RHOJ and CDC42 controls active α5β1 integrin traffic and FN fibrillogenesis. During angiogenesis, the formation and proper morphogenesis of newly formed blood vessels crucially depend on the dynamic regulation of active α5β1 integrin traffic along with FN secretion, polymerization, and turnover [7, 11]. RHOJ negatively regulates the accumulation of internalization FN-fragment-bound active α5β1 integrins in early endosomes (EEs) and their traffic towards post-Golgi carrier vesicles (PGCs), while promoting their targeting to degradative late endosomes/lysosomes (LEs/LYs) [8]. The accumulation of internalized active α5β1 integrins upon RHOJ silencing in ECs may be due to the loss of RHOJ-driven inhibition of endocytosis

from the plasma membrane and/or stimulation of degradation in LEs/LYs. The lack of RHOJ favors active α5β1 integrin localization in PGCs [8]. Hence, RHOJ may act at the trafficking junction where internalized active α5β1 integrins are directed either to PGCs, from which they recycle, or to LEs/LYs, where they can be degraded; RHOJ may inhibit the former and promote the latter pathway. CDC42, which, *via* FMNL3, promotes the PGC-mediated anterograde traffic of secretory cargoes from the TGN to the plasma membrane [15], counteracting RHOJ inhibition of FN polymerization [8]. The mutual inhibition between CDC42 and RHOJ may depend on their competition for the binding to shared effector proteins, such as PAK3 and PAK1. Since PAK3 impacts on FN polymerization, but not active α5β1 integrin traffic [8], other CDC42 and RHOJ shared effectors, *e.g.* FMNL3, may be likely involved. RHOJ interaction and signaling *via* FMNL3 [16] is not depicted. In ECs, active α5β1 integrin recycling and basolateral FN secretion depend on transmembrane PTPRF receptor and PPFIA1 adaptor protein that localize in proximity of fibrillar adhesions [7, 11]. Endocytosed inactive α5β1 integrins are immediately recycled back to the cell surface from a RAB4-containing early endosomal compartment [11].


PAK3, PAK1, ...
RAB21
RHOJ
LAMP1
CDC42
FMNL3
LEs/LYs
EEA1
EEs
RAB11B
TGN46
PGCs
PPFIA1
RAB4
EEs
TGN
Active α5β1 integrin
Inactive α5β1 integrin
PTPRF
Fibronectin dimers
Polymerized fibronectin
Blood vessel lumen
Extracellular matrix
Endothelial cell