Receptor Tyrosine Kinases Endocytosis in Endothelium
Biology and Signaling

Xi Zhang, Michael Simons

Abstract—Receptor tyrosine kinases are involved in regulation of key processes in endothelial biology, including proliferation, migration, and angiogenesis. It is now generally accepted that receptor tyrosine kinase signaling occurs intracellularly and on the plasma membrane, although many important details remain to be worked out. Endocytosis and subsequent intracellular trafficking spatiotemporally regulate receptor tyrosine kinase signaling, whereas signaling endosomes provide a platform for the compartmentalization of signaling events. This review summarizes recent advances in our understanding of endothelial receptor tyrosine kinase endocytosis and signaling using vascular endothelial growth factor receptor-2 as a paradigm. (*Arterioscler Thromb Vasc Biol*. 2014;34:1831-1837.)

Key Words: endocytosis ■ fibroblast growth factor receptor 1 ■ myosin-VI ■ neuropilin ■ synectin ■ receptor, epidermal growth factor ■ vascular endothelial growth factor receptor-2

Vascular networks reach every organ system in the body. In addition to supplying oxygen and nutrients to tissue, as well as a variety of hormones, the vasculature is able to affect function of different organs via direct paracrine secretion of various molecules directly. Endothelial cells (ECs) are the primary source of these factors and play a central role in most, if not all, kinds of biological activities, physiological or pathological, that take place in blood vessels. In addition, the endothelium plays a central role in the maintenance of normal vasculature, including regulation of its permeability, stability, and proliferation.[1–3]

Please see http://atvb.ahajournals.org/site/misc/ ATVB_in_Focus.xhtml for all articles published in this series.

ECs respond to a variety of biochemical and mechanical inputs via several different receptor systems. Among these, receptor tyrosine kinases (RTKs), such as vascular endothelial growth factor receptor-2 (VEGFR2) and fibroblast growth factor receptor-1 (FGFR1), are essential regulators of such essential EC activities as proliferation, migration, angiogenesis, and vascular permeability. Although traditionally RTKs are thought to signal from the plasma membrane, recent studies have demonstrated that several critical signaling events occur in the cytoplasm, after RTK endocytosis, which bring them into close proximity with either downstream signaling targets or phosphatases.[4] Interestingly, the extent to which a specific RTK's signaling is affected by endocytosis is different for different receptors. This could be because of different endocytosis pathways or intracellular trafficking routes that these receptors take (Figure 1).

To date, epidermal growth factor receptor (EGFR) has been one of the best-studied receptors in regard to the connection between its trafficking and signaling.[5] In addition to plasma membrane signaling, much of EGFR signaling output occurs from various endocytic compartments because its uptake and intracellular trafficking brings EGFR into association with a series of adaptor proteins involved in various signaling cascade.[6] In particular, EGFR activates extracellular signal-regulated kinases 1 and 2 (ERK1/2)[7] and AKT[8] signaling from EEA1-positive (early endosome antigen 1) early endosomes.

Endocytosis of EGFR, as well as of other receptors, is regulated by the Rab family proteins.[9] The Rabs (Ras-related proteins in brain) are GTP-binding proteins that switch between active GTP-bound and inactive GDP-bound forms. The switch between the 2 states is mediated by guanine nucleotide exchange factors and GTPase-activating proteins. The Rab proteins regulate various stages of cellular transport, including vesicle budding, endosome trafficking, and tethering of vesicles with target membranes.[10] Different Rab proteins share structural similarity but have unique subcellular localization and effector proteins, which enable them to be involved in different stages of cellular transport.[9]

Here, we review the recent progress in the understanding of endocytosis and signaling of the 2 endothelial RTKs, VEGFR2 and FGFR1, and the effect this process has on their signaling and biological activities.

Endocytosis Pathways of VEGFR2 and FGFR1

VEGFR2 is the primary signaling receptor of VEGF-A, the endothelial growth factor central to much of the blood vessel

Nonstandard Abbreviations and Acronyms	
CCVs	clathrin-coated vesicles
EC	endothelial cell
EGFR	epidermal growth factor receptor
ERK1/2	extracellular signal-regulated kinases 1 and 2
FGFR1	fibroblast growth factor receptor-1
PTPs	protein tyrosine phosphatases
RTK	receptor tyrosine kinases
VEGFR2	vascular endothelial growth factor receptor-2

biology, as well as a related ligand VEGF-C. After binding to its ligands, VEGFR2 undergoes dimerization and activation characterized by phosphorylation of key tyrosine residues in its cytoplasmic domain.[11] This is followed by endocytosis via the clathrin/dynamin-mediated pathway.[12] In addition to this ligand-dependent endocytosis, 2 other forms of VEGFR2 endocytosis have also been described: a slow constitutive recycling and shear stress–induced VEGFR2 endocytosis.

In unstimulated ECs, VEGFR2 is located both on the plasma membrane and in EEA1-positive early endosomes, where its presence is thought to be because of constitutive endocytosis.[13] The endosomal population of VEGFR2 undergoes short-loop Rab4-dependent recycling to the plasma membrane, rather than long-loop Rab11-dependent recycling.[14] The function of the constitutive VEGFR2 endocytosis and recycling remains unclear. Interestingly, ligand-independent VEGFR2 activation has been reported to occur in the Golgi of ECs in response to hyperglycemia.[15] Further investigation of the trafficking pathway involved could help our understanding of ligand-independent VEGFR2 functions.

Shear stress induces VEGFR2 endocytosis via phosphorylation of CD31 (platelet endothelial cell adhesion molecule [PECAM]), a transmembrane protein thought to be involved in shear stress sensing.[16] In this process, blood flow induces the formation of a VE-cadherin–β-catenin–VEGFR2 complex[17] and aforementioned PECAM phosphorylation. Once phosphorylated, PECAM facilitates VEGFR2 transactivation by a Src family kinase,[18] leading to the activation of downstream signaling events, such as ERK1/2 activation[19] and eNOS phosphorylation.[20] The shear stress–induced VEGFR2 endocytosis is caveolae dependent,[21] and the trafficking pathway involved is largely unknown.

Ligand-activated (VEGF) VEGFR2 endocytosis and subsequent intracellular trafficking are a tightly controlled process. The ligand-induced autophosphorylation of the receptor's kinase domain increases the rate of its internalization[22] that proceeds via clathrin-coated pits. AP2 and other adaptor proteins are involved in the recruitment of clathrin to plasma membrane, where clathrin undergoes polymerization, forming lattice-like coat.[23] Dynamin mediates the membrane fission and budding of clathrin-coated vesicles.[24] After internalization, the clathrin lattice is disassembled, leaving the uncoated vesicle to traffic toward endosomes.[23] VEGFR2 undergoes Rab5-dependent trafficking to early endosomes.[14,25] In an unusual departure from conventional endosomal trafficking, VEGFR2 trafficking is in part dependent on the synectin–myosin-VI complex (Figure 2A) because deletion of either molecule causes delayed VEGFR2 appearance in EEA1-positive early endosomes.[26]

Synectin (GIPC1) is a single domain PDZ protein that can bind to several transmembrane proteins and receptors, including another VEGF-A receptor neuropilin-1, nerve growth factor receptor TrkA,[13] nontyrosine kinase fibroblast growth factors receptor Syndecan-4,[27] and α5 and α6 integrins[28] among others. In addition to the PDZ domain, synectin also has the ability to bind intracellular motor protein myosin-VI (Figure 2B). Myosin-VI is a unique member of the myosin family because of its ability to move toward the minus end of actin filaments. This allows it to move cargo from the plasma membrane into the cell along actin filaments. Myosin-VI plays a role in different stages of endocytosis, such as receptor internalization and sorting, through interaction with different binding partners.[29] Synectin, which associates with peripheral endocytic vesicles, binds myosin-VI and facilitates trafficking toward early endosomes.[30] The association between synectin and myosin-VI has been demonstrated in neurons[31] and epithelial cells,[32–34] besides ECs.[26,35]

The recruitment of the synectin–myosin-VI complex to Rab5-positive endosomes usually involves adaptor proteins
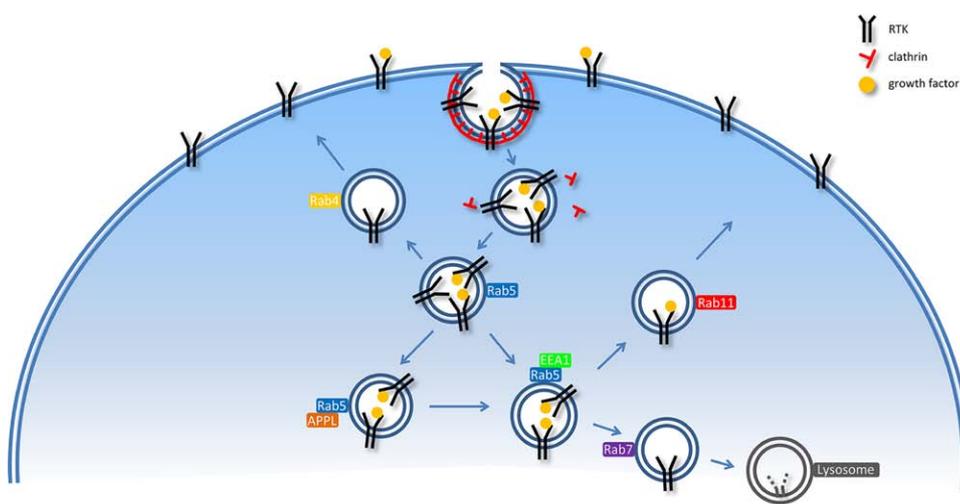

Figure 1. General receptor tyrosine kinase (RTK) endocytosis and recycling pathways. Ligand binding induces RTK activation and internalization (only clathrin-mediated pathway is shown). Internalized RTK undergoes trafficking to Rab5-positive endosomes, where they are sorted for either degradation or recycling. The degradation pathway is mediated by Rab7, whereas the recycling pathway is dependent on Rab4 (short-loop) or Rab11 (long-loop). Specific details may vary for each particular RTK. APPL indicates adaptor protein containing PH domain, PTB domain and Leucine zipper motif; and EEA1, early endosome antigen 1.
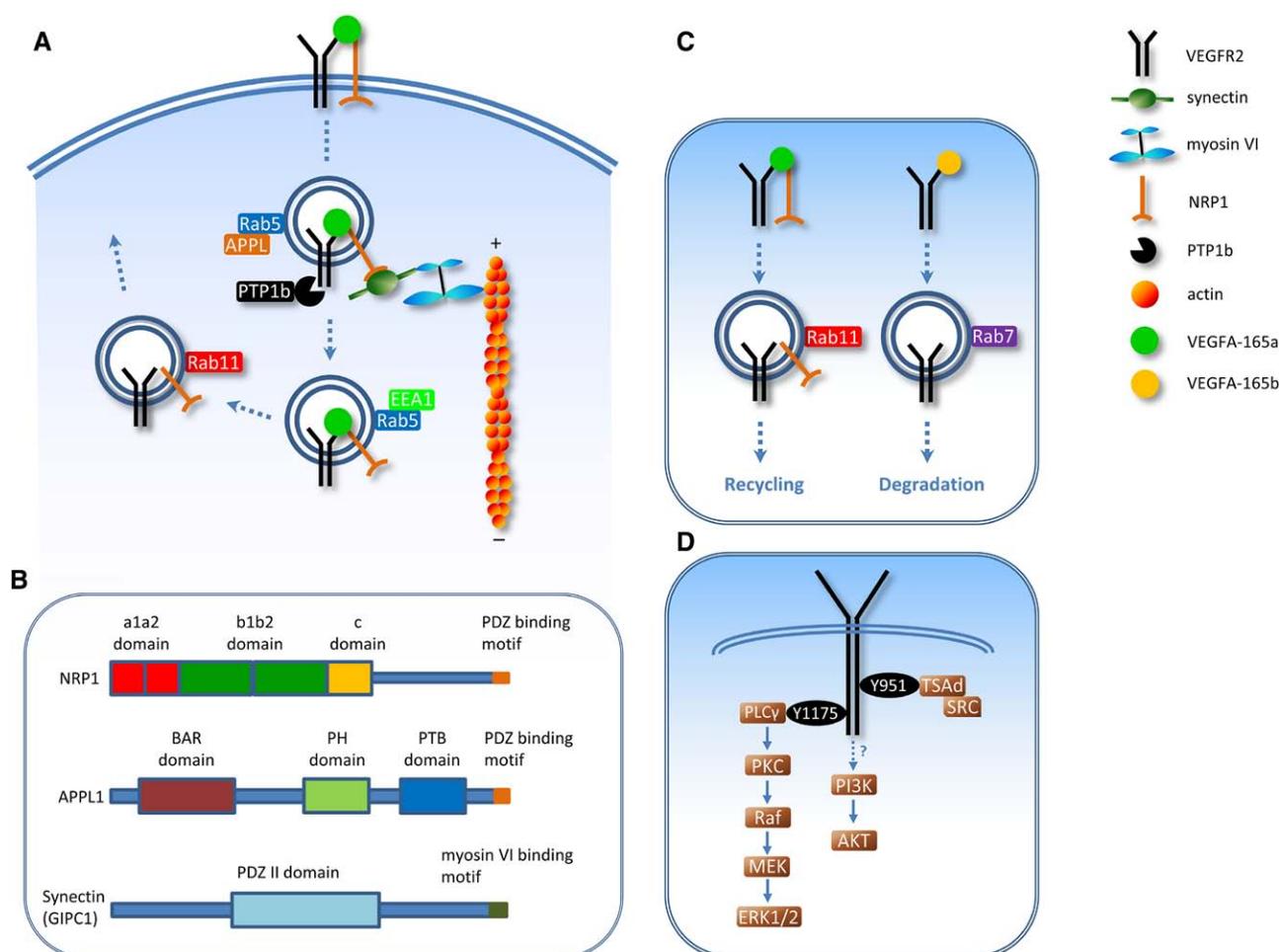
Figure 2. Vascular endothelial growth factor receptor-2 (VEGFR2) endocytosis and recycling pathways. **A**, VEGFR2 trafficking requires the synectin–myosin-VI complex, which is recruited by neuropilin-1 (NRP1) after receptor internalization. **B**, The PDZ binding motif on NRP1 and APPL1 mediates binding to the PDZ domain of synectin. Synectin forms a complex with myosin-VI via its myosin-VI binding motif. **C**, The internalized VEGFR2–NRP1 receptor complex undergoes Rab11-dependent recycling. In the absence of NRP1, VEGFR2 is degraded via Rab7-positive late endosome. **D**, VEGFR2: Src activation is downstream of phosphorylation of tyrosine 951 and extracellular signal-regulated kinases 1 and 2 (ERK1/2) activation is downstream of phosphorylation of tyrosine 1175. The mechanism of VEGF-induced AKT activation is unclear. PTP indicates protein tyrosine phosphatases; and TSAd, T-cell–specific adaptor. APPL indicates adaptor protein containing PH domain, PTB domain and Leucine zipper motif; BAR domain, bin–amphiphysin–Rvs domain; EEA1, early endosome antigen 1; MEK, MAPK and ERK kinase; PH domain, pleckstrin homology domain; PI3K, phosphatidylinositol 3′ kinase; PKC, protein kinase C; and PLC, phospholipase C.

APPL1 and APPL2 (adaptor protein containing PH domain, PTB domain and Leucine zipper motif).[36,37] However, in the case of VEGFR2, the role of APPL seems to be played by neuropilin-1[25] that is able to bind VEGF-A$_{165}$ via its extracellular VEGF-binding site and myosin-VI via its cytoplasmic domain (Figure 2B). In this model, VEGF-A is thought to form a complex with VEGFR2 and neuropilin-1 that is then endocytosed and trafficked together.[38]

Once in early endosomes, VEGFR2 can undergo either lysosomal degradation or recycling back to plasma membrane. About 40% of VEGFR2 is degraded within 30 minutes of VEGF stimulation[39] with the receptor sorted for lysosomal degradation via Rab7.[39] Proteasome-dependent degradation of VEGFR2 has also been reported (ie, in part, regulated by VEGFR2-mediated activation of p38-MAPK that lessens the receptor's degradation by the 26S proteasome system).[40] The remainder of endocytosed receptors undergoes recycling. Although constitutively endocytosed, VEGFR2 may recycle

in a Rab4-dependent manner, VEGF stimulation switches VEGFR2 recycling to a Rab11-dependent pathway.[14]

Neuropilin-1 plays a critical role, not only in the recruitment of synectin in VEGFR2 endocytosis but also in VEGFR2 recycling to the membrane. Stimulation of HUVECs (human umbilical vein endothelial cells) with VEGF-A$_{165}$a that binds both VEGFR2 and neuropilin-1 leads VEGFR2 to Rab11-dependent recycling, whereas stimulation with VEGF-A$_{165}$b, which does not bind neuropilin-1, causes Rab7-dependent degradation of VEGFR2 (Figure 2C).[14] In this manner, neuropilin-1 promotes both VEGFR2 endocytosis and recycling to the membrane and, therefore, relatively increases the proportion of VEGFR2 available for VEGF binding.

Similar to VEGFR2, signaling by FGFR1, the principal FGF receptor in the endothelium, is also regulated by its endocytosis.[41,42] The receptor's endocytosis can proceed via many different pathways. Depletion of E-Syt2, an endocytic adaptor protein that interacts with both activated FGFR1 and

the clathrin adaptor complex AP2, inhibits FGFR1 endocytosis and signaling in *Xenopus* embryo, suggesting clathrin-mediated pathway.[41] FGFR1 was also reported to interact with caveolin-1 and the knockdown of caveolin-1 decreases FGF2-induced activation of AKT and ERK1/2 in ovine fetoplacental artery endothelial cells.[43] Finally, in ECs, FGFR1 endocytosis can proceed via macropinocytosis.[3] Thus, it is likely that for FGFR1 the predominant mode of uptake is cell type specific.

Once internalized, FGFR1 is found in Rab5/EEA1-positive early endosomes[3,44] from where the majority of the activated receptors are sorted for degradation via Lamp1-positive late endosomes, whereas a relatively small proportion is recycled back to the plasma membrane for further stimulation.[45]

Endocytosis-Dependent Regulation of VEGFR2 Signaling

One unique aspect of VEGFR2 endocytosis is its regulation by several transmembrane and cytosolic interacting proteins, which play a role in either VEGFR2 internalization or degradation. A group of such proteins involved in VEGFR2 internalization is Dab2, ephrin-B2, and PAR-3. Dab2,[46] a clathrin-associated sorting protein, and the cell polarity regulator PAR-3 interact with the transmembrane protein ephrin-B2 and VEGFR2. Disruption of this interaction by silencing of Dab2 or PAR-3 causes reduced VEGFR2 internalization and impaired VEGF-induced angiogenesis. After RTKs are internalized into early endosomes, a proportion of the receptors is modified by ubiquitin and then sorted for lysosomal degradation. CCM3[47] and myoferlin,[48] respectively, associate with VEGFR2 in ECs and serve to enhance VEGFR2 stability by preventing receptor degradation.

Apart from internalization and degradation, VEGFR2 signaling is regulated by protein tyrosine phosphatases (PTPs), such as VE-PTP and PTP1b. VE-PTP is a transmembrane phosphatase that associates via its extracellular domain with VE-cadherin at EC–cell junctions.[49] VEGF stimulation causes the activation and translocation of VEGFR2 to the junctions where the receptor is silenced by VE-PTP. VE-PTP also abolishes tyrosine phosphorylation of VE-cadherin mediated by VEGFR2 and thus enhances endothelial cell junction integrity.[50] Silencing of VE-PTP boosts VEGFR2 signaling activity, while also increasing permeability.[51]

Unlike VE-PTP, PTP1b affects VEGFR2 signaling further away from the plasma membrane. PTP1b is an endoplasmic reticulum membrane bound phosphatase with its catalytic domain facing the cytosol.[52] The extensive endoplasmic reticulum network that reaches cell periphery enables PTP1b to come in close proximity with internalized VEGFR2 as it undergoes intracellular trafficking. Delayed trafficking of VEGFR2 to EEA1-positive endosomes observed in synectin or myosin-VI null ECs or in ECs expressing a truncated neuropilin-1 with its cytoplasmic domain deleted results in reduced VEGF-dependent activation of ERK1/2 pathway.[25,26] This can be corrected by suppression of PTP1b expression or activity, thus suggesting that the delay in VEGFR2 trafficking exposes the receptor to prolonged dephosphorylation by PTP1b.[53]

It is unclear in which subcellular compartment VEGFR2 is dephosphorylated by PTP1b. Studies of EGFR–PTP1b interactions showed that endosomes and endoplasmic reticulum form close membrane contacts, which is the possible site of contact between the 2 molecules.[54] However, a study of insulin receptor trafficking found the interaction between IR (insulin receptor) and PTP1b in a perinuclear endosomal compartment.[55] Thus, it is likely that PTP1b interacts with different RTKs in different subcellular compartment or compartment interfaces. Because RTK signaling is regulated both spatially and temporally, when and where they are deactivated by protein tyrosine phosphatases has a critical effect on the downstream signaling events. The driving force behind the formation of these compartment interfaces, and the recruitment of PTP1b to the contact sites remains to be elucidated.

Another interesting aspect of PTP1b function is its potential to regulate RTK endocytosis. The endosomal-sorting complexes required for transport-0 complex, which is composed of the ubiquitin-binding proteins STAM1 (signal transducing adaptor molecule 1) and STAM2 (signal transducing adaptor molecule 2) and their binding partner Hrs, binds to ubiquitin moieties on RTKs and facilitates the formation of multivesicular bodies which then fuse with lysosomes.[56] STAM2 is a PTP1b substrate and the latter's knockdown increases STAM2 phosphorylation,[57] which in turn influences its function and localization. Therefore by affecting STAM2 phosphorylation, PTP1b potentially affects the degradation of activated RTKs. It is unclear whether PTP1b specifically affects VEGFR2 lysosomal degradation. VEGF stimulation resulted in increased phosphorylation of Hrs and the colocalization of VEGFR2 with the HRS/STAM complex,[13] suggesting the involvement of this protein complex in VEGFR2 trafficking.

Biology of RTK Endocytosis in Endothelium

Increasing evidence from several studies showed the essential role of endocytosis in the optimal activation of RTKs and subsequent signaling events.[58,59] Endocytosis brings the receptors into close proximity of downstream targets or keeps them away from the dephosphorylation of protein tyrosine phosphatases. In general, RTKs have ≥1 downstream signaling pathway, yet the pathways are not necessarily activated at exactly the same time point, or for the same duration, or at the same subcellular localization. The relationship between RTK endocytosis and signaling requires better knowledge of the localization of their downstream targets. It is likely that each downstream signaling pathway has a unique signaling compartment along the RTK trafficking route. Such signaling compartments enable tight regulation of activation and inactivation of the signaling pathways. Knowledge on this aspect of receptor biology is still largely insufficient. Current understanding of signaling compartments regulating VEGFR2 signaling pathways is discussed below.

ERK1/2 Activation and Angiogenesis

VEGFR2 mediates EC proliferation, angiogenesis, and arteriogenesis and controls vascular lumen size via the activation of ERK1/2.[26,60,61] VEGF activates ERK1/2 through the classical Raf-MEK-ERK signaling cascade (Figure 2D).[62] FGF2 also

induces ERK1/2 activation[63] and FGF2-dependent ERK1/2 signaling plays a key role in the maintenance of VEGFR2 expression.[1] In both cases, ERK1/2 activation depends on receptor internalization because inhibition of internalization abrogates VEGF- and FGF2-induced ERK1/2 signaling.[64]

Knockdowns of Rab7 or Rab11 increase VEGF-induced ERK1/2 activation.[26] Because these interventions, respectively, cause reduced degradation or recycling of VEGFR2, this suggests that ERK1/2 signaling occurs in a subcellular compartment preceding these compartments. ERK1/2 signaling has been associated with clathrin-coated vesicles (CCVs), early endosomes, and late endosomes. Evidence for the role of CCVs and early endosomes comes from studies of neurotrophin receptor TrkA in neurons. Several signaling components of the Ras-MAPK pathway, such as Ras, C-Raf, Mek, and ERK1/2, were found to be present in CCVs in unstimulated cells and phospho-ERK1/2 was significantly enriched in CCVs after NGF (nerve growth factor) treatment.[65] On the contrary, AKT and phospho-AKT were not detectable in CCVs before or after NGF stimulation.[65] Phospho-ERK1/2 presence has also been demonstrated in Rab5-positive early endosomes using confocal microscopy, and increased phospho-ERK1/2 in Rab5-positive endosomes was observed after NGF treatment.[66] Furthermore, ERK1/2 activation was demonstrated to occur in EEA1-positive endosomes in ECs because delayed trafficking of activated VEGFR2 to this endosomal compartment caused impaired ERK1/2 activation.[25] PI3K and phospho-AKT were also present in early endosomes.[67] The detection of ERK1/2 signaling in CCVs is notable, considering the transient phase of these vesicles and rapid assembly and disassembly of clathrin coats in the vesicles, making CCVs a seemingly unsuitable compartment for signaling events to take place.

Late endosome involvement in ERK1/2 signaling was reported in studies of EGFR signaling. The MEK1 partner protein MP1 and the late endosomal adaptor protein p14 form a scaffold complex that recruits MAPK to late endosomes.[68] On EGF stimulation, activated ERK1/2 was found to colocalize with p14 and MP1 on late endosomes. RNAi-mediated silencing of MP1 or p14 caused reduced EGF-induced ERK1/2 activation.[69] Taken together, ERK1/2 signaling is found to span a variety of subcellular compartments, from CCVs to late endosomes.

AKT Activation and EC Survival

VEGF promotes EC survival or antiapoptotic signaling pathways via the antiapoptotic kinase AKT/PKB (Figure 2D). Several studies have suggested that AKT activation requires VEGFR2 internalization and endocytosis to a similar extent as ERK1/2 activation.[26,70,71] As discussed above, AKT activation was not detected in clathrin-coated vesicles in studies of neurotrophin receptor TrkA in neurons.[65] Interestingly, in the context of EGFR signaling, AKT, by immunostaining, has been shown to be closely associated with 2 Rab5 effectors, APPL1 and APPL2,[72] which are located on a subset of Rab5-positive early endosomes.[36] A knockdown of APPL1 in zebrafish led to a strongly diminished IGF (insulin-like growth factor)-induced AKT phosphorylation, whereas IGF-induced ERK1/2 phosphorylation remained largely unchanged.[72] This is in

agreement with the findings that ERK1/2 signals in endosomal compartments other than just early endosomes. Therefore, APPL1 depletion has a minimal effect on IGF-induced ERK1/2 activation. On the contrary, IGF-induced AKT signaling is confined to Rab5/APPL-positive endosomes. APPL1 is also involved in regulating signaling of EGFR,[36] TrkA,[13] and adiponectin receptors.[73] It is unclear whether APPL1 also regulates VEGFR2 endocytosis and signaling because neuropilin-1 is the molecule in ECs that mediates the recruitment of the synectin–myosin-VI complex to activated VEGFR2. It remains to be elucidated whether VEGFR2-induced AKT activation depends on APPL1 and APPL2, and whether the signaling endosome for AKT is receptor specific.

Src Activation, EC Migration, and Vascular Permeability

Activated VEGFR2 recruits the signaling adaptor T-cell–specific adaptor, which promotes Src activation (Figure 2D). Src mediates Src-FAK (focal adhesion kinase) signaling activity, which is involved in EC migration and vascular permeability.[74] The process of cell migration includes cell polarization, lamellipodia formation, and the assembly and disassembly of focal adhesions, whereas vascular permeability is associated with the integrity of EC junctions. Both types of activities involve signaling events in specialized compartments of ECs and the endosomal trafficking, and signaling events in these contexts are different from what is discussed above. Inactive Src resides in the perinuclear region[75] which, according to other studies,[76] is likely to be Rab7-positive late endosomes. In contrast, active Src is not observed in the perinuclear region, suggesting rapid translocation on activation.[76] Once activated, Src moves to focal adhesions or cell–cell contact sites[39] through the Rab11-dependent recycling pathway[75] or the endosomal-sorting complexes required for transport pathway[71] according to different studies. Src becomes more active as it progresses from the perinuclear region to the plasma membrane, demonstrating an activation gradient along the trafficking route.[75] This finding suggests that Src activation only begins after it exits late endosomes and enters the recycling endosome. When compared with ERK1/2 and AKT, which are activated in early endosomes on VEGF stimulation, Src is activated in a later signaling compartment, probably on the VEGFR2 recycling route.

Conclusions

There has been tremendous progress in recent years in studies of spatial and temporal aspects of RTK signaling. It is now clear that RTK endocytosis and signaling are closely related events. Endocytosis regulates signaling spatiotemporally as cytoplasmic uptake and trafficking bring activated RTKs into close proximity to downstream signaling molecules and signaling regulators, such as protein tyrosine phosphatases. Because each RTK has several downstream signaling targets, each signaling pathway has a unique signaling endosome or signaling compartment. It is further likely that these differ for different RTKs. Such setting allows for spatial or temporal separation of individual signaling pathway and to a considerable extent determines specificity of each RTK signaling.

In ECs, current evidence suggests that VEGFR2 undergoes VEGF-induced endocytosis via clathrin-mediated pathway,[77] whereas FGF2-induced FGFR1 endocytosis is clathrin independent.[3,43] Although ERK1/2 activation has been reported to occur in multiple endosomal compartments, the bulk of signaling activity is likely to originate from Rab5/EEA1-positive early endosomes in ECs.[25,26] It is likely that VEGFR2, FGFR1, and other receptors have different downstream signaling endosomes. Additional studies are needed to unravel signaling mechanisms of endothelial RTK fully.

Sources of Funding

Disclosures

None.

References

1. Murakami M, Nguyen LT, Hatanaka K, Schachterle W, Chen PY, Zhuang ZW, Black BL, Simons M. FGF-dependent regulation of VEGF receptor 2 expression in mice. *J Clin Invest*. 2011;121:2668–2678.
2. Murakami M, Nguyen LT, Zhuang ZW, Zhang ZW, Moodie KL, Carmeliet P, Stan RV, Simons M. The FGF system has a key role in regulating vascular integrity. *J Clin Invest*. 2008;118:3355–3366.
3. Elfenbein A, Lanahan A, Zhou TX, Yamasaki A, Tkachenko E, Matsuda M, Simons M. Syndecan 4 regulates FGFR1 signaling in endothelial cells by directing macropinocytosis. *Sci Signal*. 2012;5:ra36.
4. Sorkin A, von Zastrow M. Endocytosis and signalling: intertwining molecular networks. *Nat Rev Mol Cell Biol*. 2009;10:609–622.
5. Hupalowska A, Miaczynska M. The new faces of endocytosis in signaling. *Traffic*. 2012;13:9–18.
6. Sorkin A, Goh LK. Endocytosis and intracellular trafficking of ErbBs. *Exp Cell Res*. 2009;315:683–696.
7. Kranenburg O, Verlaan I, Moolenaar WH. Dynamin is required for the activation of mitogen-activated protein (MAP) kinase by MAP kinase kinase. *J Biol Chem*. 1999;274:35301–35304.
8. Wang Y, Pennock S, Chen X, Wang Z. Endosomal signaling of epidermal growth factor receptor stimulates signal transduction pathways leading to cell survival. *Mol Cell Biol*. 2002;22:7279–7290.
9. Ceresa BP. Regulation of EGFR endocytic trafficking by rab proteins. *Histol Histopathol*. 2006;21:987–993.
10. Zerial M, McBride H. Rab proteins as membrane organizers. *Nat Rev Mol Cell Biol*. 2001;2:107–117.
11. Koch S, Tugues S, Li X, Gualandi L, Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. *Biochem J*. 2011;437:169–183.
12. Pasula S, Cai X, Dong Y, et al. Endothelial epsin deficiency decreases tumor growth by enhancing VEGF signaling. *J Clin Invest*. 2012;122:4424–4438.
13. Ewan LC, Jopling HM, Jia H, Mittar S, Bagherzadeh A, Howell GJ, Walker JH, Zachary IC, Ponnambalam S. Intrinsic tyrosine kinase activity is required for vascular endothelial growth factor receptor 2 ubiquitination, sorting and degradation in endothelial cells. *Traffic*. 2006;7:1270–1282.
14. Ballmer-Hofer K, Andersson AE, Ratcliffe LE, Berger P. Neuropilin-1 promotes VEGFR-2 trafficking through Rab11 vesicles thereby specifying signal output. *Blood*. 2011;118:816–826.
15. Warren CM, Ziyad S, Briot A, Der A, Iruela-Arispe ML. A ligand-independent VEGFR2 signaling pathway limits angiogenic responses in diabetes. *Sci Signal*. 2014;7:ra1.
16. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature*. 2005;437:426–431.
17. Shay-Salit A, Shushy M, Wolfovitz E, Yahav H, Breviario F, Dejana E, Resnick N. VEGF receptor 2 and the adherens junction as a mechanical transducer in vascular endothelial cells. *Proc Natl Acad Sci U S A*. 2002;99:9462–9467.
18. Chiu YJ, McBeath E, Fujiwara K. Mechanotransduction in an extracted cell model: Fyn drives stretch- and flow-elicited PECAM-1 phosphorylation. *J Cell Biol*. 2008;182:753–763.
19. Tai LK, Zheng Q, Pan S, Jin ZG, Berk BC. Flow activates ERK1/2 and endothelial nitric oxide synthase via a pathway involving PECAM1, SHP2, and Tie2. *J Biol Chem*. 2005;280:29620–29624.
20. Jin ZG, Wong C, Wu J, Berk BC. Flow shear stress stimulates Gab1 tyrosine phosphorylation to mediate protein kinase B and endothelial nitric-oxide synthase activation in endothelial cells. *J Biol Chem*. 2005;280:12305–12309.
21. Yu J, Bergaya S, Murata T, Alp IF, Bauer MP, Lin MI, Drab M, Kurzchalia TV, Stan RV, Sessa WC. Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodeling of blood vessels. *J Clin Invest*. 2006;116:1284–1291.
22. Dougher M, Terman BI. Autophosphorylation of KDR in the kinase domain is required for maximal VEGF-stimulated kinase activity and receptor internalization. *Oncogene*. 1999;18:1619–1627.
23. McMahon HT, Boucrot E. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. *Nat Rev Mol Cell Biol*. 2011;12:517–533.
24. Schmid SL, Frolov VA. Dynamin: functional design of a membrane fission catalyst. *Annu Rev Cell Dev Biol*. 2011;27:79–105.
25. Lanahan A, Zhang X, Fantin A, Zhuang Z, Rivera-Molina F, Speichinger K, Prahst C, Zhang J, Wang Y, Davis G, Toomre D, Ruhrberg C, Simons M. The neuropilin 1 cytoplasmic domain is required for VEGF-A-dependent arteriogenesis. *Dev Cell*. 2013;25:156–168.
26. Lanahan AA, Hermans K, Claes F, Kerley-Hamilton JS, Zhuang ZW, Giordano FJ, Carmeliet P, Simons M. VEGF receptor 2 endocytic trafficking regulates arterial morphogenesis. *Dev Cell*. 2010;18:713–724.
27. Gao Y, Li M, Chen W, Simons M. Synectin, syndecan-4 cytoplasmic domain binding PDZ protein, inhibits cell migration. *J Cell Physiol*. 2000;184:373–379.
28. Tani TT, Mercurio AM. PDZ interaction sites in integrin alpha subunits. T14853, TIP/GIPC binds to a type I recognition sequence in alpha 6A/alpha 5 and a novel sequence in alpha 6B. *J Biol Chem*. 2001;276:36535–36542.
29. Tumbarello DA, Kendrick-Jones J, Buss F. Myosin VI and its cargo adaptors - linking endocytosis and autophagy. *J Cell Sci*. 2013;126(Pt 12):2561–2570.
30. Buss F, Kendrick-Jones J. How are the cellular functions of myosin VI regulated within the cell? *Biochem Biophys Res Commun*. 2008;369:165–175.
31. Yano H, Ninan I, Zhang H, Milner TA, Arancio O, Chao MV. BDNF-mediated neurotransmission relies upon a myosin VI motor complex. *Nat Neurosci*. 2006;9:1009–1018.
32. Aschenbrenner L, Lee T, Hasson T. Myo6 facilitates the translocation of endocytic vesicles from cell peripheries. *Mol Biol Cell*. 2003;14:2728–2743.
33. Naccache SN, Hasson T, Horowitz A. Binding of internalized receptors to the PDZ domain of GIPC/synectin recruits myosin VI to endocytic vesicles. *Proc Natl Acad Sci U S A*. 2006;103:12735–12740.
34. Reed BC, Cefalu C, Bellaire BH, Cardelli JA, Louis T, Salamon J, Bloecher MA, Bunn RC. GLUT1CBP(TIP2/GIPC1) interactions with GLUT1 and myosin VI: evidence supporting an adapter function for GLUT1CBP. *Mol Biol Cell*. 2005;16:4183–4201.
35. Valdembri D, Caswell PT, Anderson KI, Schwarz JP, König I, Astanina E, Caccavari F, Norman JC, Humphries MJ, Bussolino F, Serini G. Neuropilin-1/GIPC1 signaling regulates alpha5beta1 integrin traffic and function in endothelial cells. *PLoS Biol*. 2009;7:e25.
36. Miaczynska M, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S, Habermann B, Wilm M, Parton RG, Zerial M. APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment. *Cell*. 2004;116:445–456.
37. Varsano T, Dong MQ, Niesman I, Gacula H, Lou X, Ma T, Testa JR, Yates JR 3rd, Farquhar MG. GIPC is recruited by APPL to peripheral TrkA endosomes and regulates TrkA trafficking and signaling. *Mol Cell Biol*. 2006;26:8942–8952.
38. Eichmann A, Simons M. VEGF signaling inside vascular endothelial cells and beyond. *Curr Opin Cell Biol*. 2012;24:188–193.
39. Gampel A, Moss L, Jones MC, Brunton V, Norman JC, Mellor H. VEGF regulates the mobilization of VEGFR2/KDR from an intracellular endothelial storage compartment. *Blood*. 2006;108:2624–2631.
40. Meyer RD, Srinivasan S, Singh AJ, Mahoney JE, Gharahassanlou KR, Rahimi N. PEST motif serine and tyrosine phosphorylation controls vascular endothelial growth factor receptor 2 stability and downregulation. *Mol Cell Biol*. 2011;31:2010–2025.
41. Jean S, Mikryukov A, Tremblay MG, Baril J, Guillou F, Bellenfant S, Moss T. Extended-synaptotagmin-2 mediates FGF receptor endocytosis and ERK activation in vivo. *Dev Cell*. 2010;19:426–439.

42. Sandilands E, Akbarzadeh S, Vecchione A, McEwan DG, Frame MC, Heath JK. Src kinase modulates the activation, transport and signalling dynamics of fibroblast growth factor receptors. *EMBO Rep*. 2007;8:1162–1169.

43. Feng L, Liao WX, Luo Q, Zhang HH, Wang W, Zheng J, Chen DB. Caveolin-1 orchestrates fibroblast growth factor 2 signaling control of angiogenesis in placental artery endothelial cell caveolae. *J Cell Physiol*. 2012;227:2480–2491.

44. Bryant DM, Wylie FG, Stow JL. Regulation of endocytosis, nuclear translocation, and signaling of fibroblast growth factor receptor 1 by E-cadherin. *Mol Biol Cell*. 2005;16:14–23.

45. Irschick R, Trost T, Karp G, Hausott B, Auer M, Claus P, Klimaschewski L. Sorting of the FGF receptor 1 in a human glioma cell line. *Histochem Cell Biol*. 2013;139:135–148.

46. Nakayama M, Nakayama A, van Lessen M, Yamamoto H, Hoffmann S, Drexler HC, Itoh N, Hirose T, Breier G, Vestweber D, Cooper JA, Ohno S, Kaibuchi K, Adams RH. Spatial regulation of VEGF receptor endocytosis in angiogenesis. *Nat Cell Biol*. 2013;15:249–260.

47. He Y, Zhang H, Yu L, Gunel M, Boggon TJ, Chen H, Min W. Stabilization of VEGFR2 signaling by cerebral cavernous malformation 3 is critical for vascular development. *Sci Signal*. 2010;3:ra26.

48. Bernatchez PN, Acevedo L, Fernandez-Hernando C, Murata T, Chalouni C, Kim J, Erdjument-Bromage H, Shah V, Gratton JP, McNally EM, Tempst P, Sessa WC. Myoferlin regulates vascular endothelial growth factor receptor-2 stability and function. *J Biol Chem*. 2007;282:30745–30753.

49. Nawroth R, Poell G, Ranft A, Kloep S, Samulowitz U, Fachinger G, Golding M, Shima DT, Deutsch U, Vestweber D. VE-PTP and VE-cadherin ectodomains interact to facilitate regulation of phosphorylation and cell contacts. *EMBO J*. 2002;21:4885–4895.

50. Hayashi M, Majumdar A, Li X, et al. VE-PTP regulates VEGFR2 activity in stalk cells to establish endothelial cell polarity and lumen formation. *Nat Commun*. 2013;4:1672.

51. Mellberg S, Dimberg A, Bahram F, Hayashi M, Rennel E, Ameur A, Westholm JO, Larsson E, Lindahl P, Cross MJ, Claesson-Welsh L. Transcriptional profiling reveals a critical role for tyrosine phosphatase VE-PTP in regulation of VEGFR2 activity and endothelial cell morphogenesis. *FASEB J*. 2009;23:1490–1502.

52. Hernández MV, Sala MG, Balsamo J, Lilien J, Arregui CO. ER-bound PTP1B is targeted to newly forming cell-matrix adhesions. *J Cell Sci*. 2006;119(Pt 7):1233–1243.

53. Zhang X, Lanahan AA, Simons M. VEGFR2 trafficking: speed doesn't kill. *Cell Cycle*. 2013;12:2163–2164.

54. Eden ER, White IJ, Tsapara A, Futter CE. Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction. *Nat Cell Biol*. 2010;12:267–272.

55. Romsicki Y, Reece M, Gauthier JY, Asante-Appiah E, Kennedy BP. Protein tyrosine phosphatase-1B dephosphorylation of the insulin receptor occurs in a perinuclear endosome compartment in human embryonic kidney 293 cells. *J Biol Chem*. 2004;279:12868–12875.

56. Abella JV, Park M. Breakdown of endocytosis in the oncogenic activation of receptor tyrosine kinases. *Am J Physiol Endocrinol Metab*. 2009;296:E973–E984.

57. Stuible M, Abella JV, Feldhammer M, Nossov M, Sangwan V, Blagoev B, Park M, Tremblay ML. PTP1B targets the endosomal sorting machinery: dephosphorylation of regulatory sites on the endosomal sorting complex required for transport component STAM2. *J Biol Chem*. 2010;285:23899–23907.

58. Polo S, Di Fiore PP. Endocytosis conducts the cell signaling orchestra. *Cell*. 2006;124:897–900.

59. von Zastrow M, Sorkin A. Signaling on the endocytic pathway. *Curr Opin Cell Biol*. 2007;19:436–445.

60. Mavria G, Vercoulen Y, Yeo M, Paterson H, Karasarides M, Marais R, Bird D, Marshall CJ. ERK-MAPK signaling opposes Rho-kinase to promote endothelial cell survival and sprouting during angiogenesis. *Cancer Cell*. 2006;9:33–44.

61. Wheeler-Jones C, Abu-Ghazaleh R, Cospedal R, Houliston RA, Martin J, Zachary I. Vascular endothelial growth factor stimulates prostacyclin production and activation of cytosolic phospholipase A2 in endothelial cells via p42/p44 mitogen-activated protein kinase. *FEBS Lett*. 1997;420:28–32.

62. Takahashi T, Ueno H, Shibuya M. VEGF activates protein kinase C-dependent, but Ras-independent Raf-MEK-MAP kinase pathway for DNA synthesis in primary endothelial cells. *Oncogene*. 1999;18:2221–2230.

63. Ichise T, Yoshida N, Ichise H. FGF2-induced Ras-MAPK signalling maintains lymphatic endothelial cell identity by upregulating endothelial-cell-specific gene expression and suppressing TGFβ signalling through Smad2. *J Cell Sci*. 2014;127(Pt 4):845–857.

64. Gourlaouen M, Welti JC, Vasudev NS, Reynolds AR. Essential role for endocytosis in the growth factor-stimulated activation of ERK1/2 in endothelial cells. *J Biol Chem*. 2013;288:7467–7480.

65. Howe CL, Valletta JS, Rusnak AS, Mobley WC. NGF signaling from clathrin-coated vesicles: evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway. *Neuron*. 2001;32:801–814.

66. Delcroix JD, Valletta JS, Wu C, Hunt SJ, Kowal AS, Mobley WC. NGF signaling in sensory neurons: evidence that early endosomes carry NGF retrograde signals. *Neuron*. 2003;39:69–84.

67. Kuruvilla R, Ye H, Ginty DD. Spatially and functionally distinct roles of the PI3-K effector pathway during NGF signaling in sympathetic neurons. *Neuron*. 2000;27:499–512.

68. Teis D, Taub N, Kurzbauer R, Hilber D, de Araujo ME, Erlacher M, Offterdinger M, Villunger A, Geley S, Bohn G, Klein C, Hess MW, Huber LA. p14-MP1-MEK1 signaling regulates endosomal traffic and cellular proliferation during tissue homeostasis. *J Cell Biol*. 2006;175:861–868.

69. Teis D, Wunderlich W, Huber LA. Localization of the MP1-MAPK scaffold complex to endosomes is mediated by p14 and required for signal transduction. *Dev Cell*. 2002;3:803–814.

70. Wang Y, Nakayama M, Pitulescu ME, Schmidt TS, Bochenek ML, Sakakibara A, Adams S, Davy A, Deutsch U, Lüthi U, Barberis A, Benjamin LE, Mäkinen T, Nobes CD, Adams RH. Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. *Nature*. 2010;465:483–486.

71. Sawamiphak S, Seidel S, Essmann CL, Wilkinson GA, Pitulescu ME, Acker T, Acker-Palmer A. Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis. *Nature*. 2010;465:487–491.

72. Schenck A, Goto-Silva L, Collinet C, Rhinn M, Giner A, Habermann B, Brand M, Zerial M. The endosomal protein Appl1 mediates Akt substrate specificity and cell survival in vertebrate development. *Cell*. 2008;133:486–497.

73. Mao X, Kikani CK, Riojas RA, Langlais P, Wang L, Ramos FJ, Fang Q, Christ-Roberts CY, Hong JY, Kim RY, Liu F, Dong LQ. APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nat Cell Biol*. 2006;8:516–523.

74. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. *Nat Rev Mol Cell Biol*. 2006;7:359–371.

75. Sandilands E, Cans C, Fincham VJ, Brunton VG, Mellor H, Prendergast GC, Norman JC, Superti-Furga G, Frame MC. RhoB and actin polymerization coordinate Src activation with endosome-mediated delivery to the membrane. *Dev Cell*. 2004;7:855–869.

76. Kasahara K, Nakayama Y, Kihara A, Matsuda D, Ikeda K, Kuga T, Fukumoto Y, Igarashi Y, Yamaguchi N. Rapid trafficking of c-Src, a non-palmitoylated Src-family kinase, between the plasma membrane and late endosomes/lysosomes. *Exp Cell Res*. 2007;313:2651–2666.

77. Horowitz A, Seerapu HR. Regulation of VEGF signaling by membrane traffic. *Cell Signal*. 2012;24:1810–1820.

Significance

This review focuses on the endocytosis of specific receptor tyrosine kinases in endothelial cells and summarizes, in particular, the current knowledge about vascular endothelial growth factor receptor-2 endocytosis and signaling. The rapidly changing understanding of how this key endothelial receptor tyrosine kinase operates provides new insights into biology of key endothelium-dependent processes, such as angiogenesis and arteriogenesis, and offers the possibility of new therapeutic approaches to some of the most intractable cardiovascular illnesses currently.

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

Receptor Tyrosine Kinases Endocytosis in Endothelium: Biology and Signaling
Xi Zhang and Michael Simons

Arterioscler Thromb Vasc Biol. 2014;34:1831-1837; originally published online June 12, 2014;
doi: 10.1161/ATVBAHA.114.303217
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/34/9/1831

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the
Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for
which permission is being requested is located, click Request Permissions in the middle column of the Web
page under Services. Further information about this process is available in the Permissions and Rights
Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online
at:
http://atvb.ahajournals.org//subscriptions/