parmodulins are working will not only shed light on these compounds but also help to unravel the complex regulation of PAR1 activation on endothelial cells. In summary, the study by Aisiku and colleagues advances the possibilities of targeting PAR1 by selectively inhibiting specific pathways. This study demonstrates the value of nontraditional compounds that are directed away from the ligand-binding site. This strategy may have important implications for allosteric modulators of GPCRs in general.

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● ● ● VASCULAR BIOLOGY

Comment on Liao et al, page 1995

You've got to be kindlin!

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In this issue of *Blood*, Liao et al report that kindlin-2 is necessary for angiogenic sprouting in vitro and for developmental and tumor angiogenesis in vivo.[1] The process of blood vessel sprouting is known to involve the αVβ3 vitronectin integrin on endothelium. Kindlin-2 linkage to the C-terminal tail of β3 completed the outside-in circuit necessary for integrin signaling that is critical for navigation of new vessel sprouts.

In 1954, Theresa Kindler first reported a syndrome associated with skin blistering that was caused by an autosomal recessive mutation, which was subsequently discovered to be in the gene producing kindlin-1.[2] Two additional isoforms, kindlin-2 and kindlin-3, constitute a family of evolutionarily- and structurally-conserved proteins that are critical for integrin adhesion and signaling functions.[3] The mechanism involves the localization of kindlins, which are highly-conserved ancient adaptor proteins that bridge the cytoskeleton to integrins via their FERM domains.[4] A rare mutation in another kindlin (-3) was found to be the culprit in leukocyte-adhesion deficiency type III, a disease characterized by defects in leukocyte and platelet β_1-, β_2-, and β_3-integrin functions.[5] Interestingly, there are no human diseases known to be associated with genetic defects in kindlin-2, which is probably related to its wide tissue distribution and the fact that its deletion in animal models is embryonic lethal. Consequently, there is limited information on the physiologic functions of kindlin-2 in humans. Data derived from the study of embryonic stem cells from knockout animal models and transgenic cell lines suggest that kindlin-2 directs integrin functions during embryogenesis, but its specific functions in somatic cells such as fibroblasts, endothelium, and epithelium remained elusive.[6]

β3-integrins provide key functions from conception to death in all mammals. For example, they are critical for generation of complex body patterns during development and, with age, they play a central role in platelet function and tumor growth. When paired with the αv subunit, the αvβ3 heterodimer is expressed at low levels in quiescent vasculature and is upregulated in vasculature associated with solid tumors.[7] In fact, β3-integrin knockout mice exhibit enhanced tumor growth, and there is controversy about whether αvβ3 functions as a pro- or antiangiogenic receptor in mediating adhesion and migration of proliferative endothelial cells. Activation to shift conformation from a low- to a high-affinity state typically involves transmission of an inside-out signal that generates the adhesion and traction necessary for cell motility. After engagement and receptor clustering in the membrane, integrins can signal from the outside-in to trigger activation of tyrosine kinases, particularly those of the Src and Syk families. Tyrosine phosphorylation of c-Src is critical for linking αvβ3 to the cytoskeleton, but how it provides navigational cues necessary to carry out angiogenesis is unknown. Previous studies indicate that kindlin-2 plays a central role in pathologic and developmental angiogenesis, through activation of integrin αVβ3, but whether it facilitates inside-out activation or outside-in signals remains ill defined.[8]

In this issue, Liao et al adopted a strategy to determine how αvβ3 integrin regulates its bidirectional signaling to function as an effective gatekeeper of angiogenesis and tumor progression.[1] They take advantage of 2 knock-in mouse strains with deletions or mutations in the C-terminal of the β3 subunit. Swapping the terminal-3 amino acids of β1-integrin eliminated the capacity for c-Src to bind αvβ3, but kindlin-2 interaction was retained. A second β3 mutation lacking the C-terminal sequence (αVβ3ΔRGT) eliminated c-Src and kindlin-2 binding, which correlated with diminished endothelial cell migration and angiogenic sprouting. Mice bearing the β3ΔRGT mutation exhibited

defective developmental and tumor angiogenesis, establishing the in vivo significance of the work. Importantly, the capacity of αVβ3ΔRGT to bind ligands with high affinity was preserved, indicating that inside-out signaling was maintained, thus confirming a similar finding in platelets bearing αIIbβ3ΔRGT.[9] Thus, the defect in αVβ3ΔRGT endothelial cell spreading, migration, and angiogenesis could be specifically assigned to a defect in outside-in signaling. In a clever experiment, the defect in sprouting was rescued by using a conditional dimerizer to enforce the association of kindlin-2 with αVβ3ΔRGT, thus proving that the defect was caused by the failure of kindlin-2 to bind to the integrin and initiate signaling.

This work reveals the importance of outside-in signaling of αVβ3 subsequent to ligand binding in angiogenesis and highlights the requisite role of kindlin-2 to complete the bidirectional integrin-signaling circuit necessary for endothelial migration. Moreover, the diminished tumor weight in αVβ3ΔRGT mice, which correlated with a defect in endothelial migration and proliferation at the leading edge of forming blood vessels, established the pathologic significance of outside-in signaling through αVβ3. Thus far, clinical approaches delivering small-molecule antagonists or antibodies to αvβ3 as cancer therapeutics have yielded lackluster results.[7] By delving into the fundamental biological mechanism underlying bidirectional signaling of αVβ3, Liao and colleagues may point the way toward more specific antagonists that target the essential role that kindlin-2 plays in transmitting critical information from focal adhesions to mediate the force generation and biochemical signals necessary for angiogenesis.

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