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Regulation of integrin function controls angiogenic remodeling

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Vertebrates evolved a cardiovascular system capable of transporting over long distances oxygen and nutrients to the many different tissues that compose their multicellular organism.

Two distinct morphogenetic processes contribute to the development of the embryonic vasculature: vasculogenesis and angiogenesis.[1] During vasculogenesis, mesodermal cells differentiate into endothelial cell (EC) precursors (angioblasts), which proliferate and coalesce into a primitive network of homogeneously sized vessels known as primary capillary plexus. This initial capillary meshwork is then remodeled by angiogenesis into a mature and functional vascular bed comprised of arteries, capillaries, and veins.[1] Angiogenic remodeling coordinates with the establishment of blood flow and can occur through sprouting, intussusception, i.e. by internal division of the vessel lumen or vascular fusion.[1] Angiogenesis also includes penetration by sprouting of vessels into avascular regions of the embryo and recruitment of mural cells. In multicellular organisms, the formation of organs, including cardiovascular systems, depends on cell-to-cell and cell-to-extracellular matrix (ECM) adhesion regulated by soluble mediators, which act in paracrina and autocrine way, and by physical forces. Notably, compared to lower metazoans, vertebrates built on an entirely new set of adhesion-related genes involved in the development, maintenance, function, and regeneration of the vasculature, i.e. ECM proteins and their heterodimeric integrin adhesive receptors.[2] Because of the general significance of blood vessel formation to common inherited and acquired human diseases, such as cardiovascular malformations and cancer, there has been much interest in understanding its molecular mechanisms and outstanding reviews have well focused on the role of integrins in the different steps of angiogenesis and their potential for targeted therapies.[3-6]

In this review we will summarize the recent knowledge in the role of integrin function in the late phase of angiogenesis, when nascent vascular tree stabilize its finale shape.

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The final shape of the cardiovascular system results from the complementary and combinatorial interaction of several factors that control EC adhesion and movement: (i) local environmental factors, such as pO2; (ii) blood flow and fluid shear stress; (iii) genetically programmed extrinsic cues, such as bFGF, VEGF, and angiopoietin-1.[1] During the angiogenic process, ECs collectively move and change their reciprocal positions and interactions in response to the many stimuli that elicit their motility. To respond to the relative balance of activators impinging on them, ECs need to be capable of dynamically regulating their adhesive behavior both in terms of cell-to-cell and cell-to-ECM contacts. Integrins can exist in different functional states with respect to their affinity for ECM proteins, and regulation of integrin activation is crucial for their biological functions.[7,8] In the low affinity state, the extracellular domain of integrins is bent over the cell surface,[7,8] whereas the α and β cytoplasmic tails tightly interact through a juxta-membrane salt bridge between an arginine in the α subunit tail and an aspartic residue in the β subunit tail.[8,9] Interaction of PTB containing proteins, such as talin,[9,10] with the NPXY motif conserved in most β integrin subunits leads to an unclasping of α and β subunit tails[10] that associates to an extension of the extracellular domain and results in the transition of integrins to their high affinity state.[9,10] In addition, there are proteins other than talin that activate specific integrins by binding to certain α and β subunit cytoplasmic tails.[11,12] The observations that that prominent determinants of vascular remodeling (e.g. O2 tension, angiogenic growth

factors, and fluid shear stress) activate integrin-based adhesion could suggest a role for control of integrin function during angiogenesis. Indeed, in several cell types,[13] low O2 tension promotes cell adhesion to different ECM ligands via hypoxia-inducible factor (HIF), mostly by inducing integrin mRNA and protein expression at the cell surface; however, HIF-1a has been recently shown to induce in ECs a hypoxia-driven VEGF autocrine loop necessary for tumor angiogenesis.[14-15] Furthermore, angiogenic growth factors, including HGF, bFGF , and VEGF, act as positive regulators of endothelial integrin function[4,5] and in ECs migrating towards bFGF high affinity integrins are recruited to the leading edge where they promote new adhesions to support directed cell motility.[16] Moreover, it appears that flow alterations have a major influence in regulating vascular remodeling likely through shear stress that in ECs rapidly stimulates conformational activation of $\alpha v\beta 3$ followed by an increase in its binding affinity to ECM proteins.[17,18] The inside-out signaling through which positive regulators of endothelial integrin function exert their effects is still poorly characterized. VEGF, and bFGF appear to activate integrins through a phosphatidylinositol 3 kinase (PI3K)/Akt-dependent path way. However, it not clear whether PI3K is activated directly by docking of its p85 regulatory subunit on VEGF and bFGF tyrosine kinase receptors (TKRs) or via the Ras GTPase subfamily member R-Ras that localizes at ECM adhesive sites [19] and activates integrins through PI3K.[20,21] In ECs, VEGF promotes PI3K activity that in turn stimulates integrin linked kinase (ILK),[22] a cytoplasmic Ser/Thr kinase that recruits several regulators of actin dynamics at ECM adhesive sites where it also binds and activates $\beta 1$ integrins.[23]

We have previously shown that, during vascular development and experimental angiogenesis, ECs generate autocrine chemorepulsive signals of class 3 semaphorins (SEMA3) that endow the vascular system with the plasticity required for reshaping by inhibiting integrins.[24,25] Inhibitory autocrine loops of endothelial SEMA3 proteins would allow a tunable and fine modulation of integrin function, cell migration, and redirectioning during angiogenic remodeling. The vascular defects we found in sema3a null embryos somewhat phenocopy the mutation in ephrin-B2, EphB4, and EphB2/EphB3[26] that also act by modulating integrin function.[25] The receptor complex of five out of six SEMA3, SEMA3E being a notable exception, is constituted by a ligand binding and signal transducing subunit, respectively, represented by neuropilin (Nrp)-1 or 2 and type A (PlexA) or type D (PlexD) plexins.[27] The cytoplasmic domain of plexins is characterized by two interacting Ras GTPase-activating protein (GAP) domains separated by a linker region . Ligand-dependent clustering renders the receptor constitutively active in the presence of Rnd1 or Rac that, by binding to the linker region, relieves the interaction between the two RasGAP domains and unleashes plexin GAP activity.[28]

It has been found that plexins exert their enzymatic function on the integrin activating R-Ras GTPase[29,30] whose inhibition is required both for SEMA3A/ PlexinA1- and SEMA4D/PlexinB1-mediated growth cone collapse. All together, these data[24,25,29,30] support the concept that integrin-adhesive receptors are critical effector targets of SEMA/Plexin signaling. In summary, based on the observations that signaling from angiogenic growth factors , focal adhesion kinase, fluid shear stress, the Eph/ephrin system, and the SEMA/Plexin system impinge on integrins, it is tempting to speculate that pro- and anti-angiogenic cues regulate vascular morphogenesis quite generally by modulating integrin activation. Autocrine loops of integrin activator and inhibitors would set basal level of EC adhesion and responsiveness to paracrine integrin activators and inhibitors secreted by the target tissues, which needs to be vascularized, such as developing epithelia in the embryo or carcinomas and healing epithelia in the adult organism.

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References

1. Jain RK. Molecular regulation of vessel maturation. Nat Med 2003;9:685-93.
2. Hynes RO, Zhao Q. The evolution of cell adhesion, J Cell Biol 200;150:89–96.
3. Kalluri R. Basement membranes: structure, assembly and role in tumour angiogenesis. Nat Rev Cancer 2003;3:422-33.
4. Stupack DG. Integrins as a distinct subtype of dependence receptors. Cell Death Differ 2005;12:1021-30.
5. Stupack DG, Cheresh DA. Integrins and angiogenesis. Curr Top Dev Biol 2004;64:207-38.
6. Ingber DE. Mechanical signaling and the cellular response to extracellular matrix inangiogenesis and cardiovascular physiology. Circ Res 2002;91:877-87.
7. Hynes RO. Integrins: bidirectional, allosteric signaling machines, Cell 2002;110:673–87.
8. Carman CV, Springer TA. Integrin avidity regulation: are changes

in affinity and conformation underemphasized? Curr Opin Cell Biol 2003;15:547–56.
9. Qin J, Vinogradova O, Plow EF. Integrin bidirectional signaling: a molecular view. PLoS Biol 2004;2:e169.
10. Campbell ID, Ginsberg MH, The talin-tail interaction places integrin activation on FERM ground. Trends Biochem Sci 2004;29: 429–35.
11. Price LS, Bos JL. RAPL: taking the Rap in immunity. Nat Immunol 2004;5:1007–8.
12. Kolanus W, Nagel W, Schiller B, Zeitlmann L, Godar S, Stockinger H et al. αLβ 2 integrin/LFA-1 binding to ICAM- 1 induced by cytohesin-1, a cytoplasmic regulatory molecule. Cell 1996;86:233–42.
13. Cowden Dahl KD, Robertson SE, Weaver VM, Simon MC, Hypoxia-inducible factor regulates alphavbeta3 integrin cell surface expression. Mol Biol Cell 2005;16:1901–12.
14. Tang N, Wang L, Esko J, Giordano FJ, Huang Y, Gerber HP. Loss of HIF-1alpha in endothelial cells disrupt a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. Cancer Cell 2004;6: 485–95.
15. Serini G, Ambrosi D, Giraudo E, Gamba A, Preziosi L, Bussolino, Modeling the early stages of vascular network assembly. EMBO J 2003;22:1771-9.
16. Kiosses WB, Shattil SJ, Pampori N, Schwartz MA. Rac recruits high-affinity integrin alphavbeta3 to lamellipodia in endothelial cell migration. Nat Cell Biol 2001;3:316-20.
17. Burri PH, Hlushchuk R, Djonov V, Intussusceptive angiogenesis: its emergence, its characteristics, and its significance. Dev Dyn 2004;231:474–88.
18. Tzima E, del Pozo MA, Shattil SJ, Chien S, Schwartz MA. Activation of integrins in endothelial cells by fluid shear stress mediates Rho-dependent cytoskeletal alignment. EMBO J 2001;20:4639–47.
19. Furuhjelm J, Peranen J. The C-terminal end of R-Ras contains a focal adhesion targeting signal. J Cell Sci 2003;116:3729–38.
20. Zhang Z, Vuori K, Wang H, Reed JC, Ruoslahti E. Integrin activation by R-ras. Cell 1996;85:61–9.
21. Berrier AL, Mastrangelo AM, Downward J, Ginsberg M, LaFlamme SE. Activated R-ras, Rac1, PI 3-kinase and PKCepsilon can each restore cell spreading inhibited by isolated integrin beta1 cytoplasmic domains. J Cell Biol 2000;151:1549–60.
22. Tan C, Cruet-Hennequart S, Troussard A, Fazli L, Costello P, Sutton K. Regulation of tumor angiogenesis by integrin-linked kinase (ILK), Cancer Cell 2004;5:79–90.
23. Friedrich EB, Liu E, Sinha S, Cook S, Milstone DS, MacRae CA. Integrin-linked kinase regulates endothelial cell survival and vascular development, Mol Cell Biol 2004;24:8134–44.
24. Serini G, Valdembri D, Zanivan S, Morterra G, Burkhardt C, Caccavari F. Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. Nature 2003;424:391–7.
25. Serini G, Bussolino F. Common cues in vascular and axon guidance, Physiology (Bethesda) 2004;19:348–54.
26. Kullander K, Klein R, Mechanisms and functions of eph and ephrin signalling. Nat Rev Mol Cell Biol 2002;3:475–86.
27. Fujisawa H. Discovery of semaphorin receptors, neuropilin and plexin, and their functions in neural development. J Neurobiol 2004;59:24–33.
28. Negishi M, Oinuma I, Katoh H. Plexins: axon guidance and signal transduction. Cell Mol Life Sci 2005;62:1363-71.
29. Oinuma I, Katoh H, Negishi M. Molecular dissection of the semaphorin 4D receptor plexin-B1-stimulated R-Ras GTPase-activating protein activity and neurite remodeling in hippocampal neurons. J Neurosci 2004;24:11473–80.
30. Toyofuku T, Yoshida J, Sugimoto T, Zhang H, Kumanogoh A, Hori M, Kikutani H. FARP2 triggers signals for Sema3A-mediated axonal repulsion. Nat Neurosci. 2005;13.[Epub ahead of print].