Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2021 November ; 41(11): 2770–2772. doi:10.1161/ATVBAHA.121.316969.

AGGF1 Shows the $\alpha 5 \beta 1$ Integrin to Be Another *Akt*-or in a Common Angiogenesis Scene

Feilim Mac Gabhann, Brian H. Annex

Department of Biomedical Engineering, Institute for Computational Medicine, Johns Hopkins University, Baltimore, MD (F.M.G.). Vascular Biology Center and Department of Medicine, Medical College of Georgia at Augusta University, (B.H.A.).

Keywords

Editorials; amino acids; angiogenic proteins; integrins; morphogenesis; proto-oncogene proteins
c-akt

AGGF1 (angiogenic factor and G-patch and forkhead-associated domain 1) encodes the angiogenic factor with G-patch and FHA (forkhead associated) domain protein, which has been shown to be involved in multiple vascular morphogenesis processes, including vasculogenesis (the formation of blood vessels de novo and most often associated with early vascular development), angiogenesis (the growth and remodeling of blood vessels from existing vascular structures), and venous specification. Increased *AGGF1* expression is linked to Klippel-Trenaunay syndrome—a (rare) congenital vascular disease characterized by malformed venous structures.¹ Building on a series of prior publications, in this issue of *ATVB*, Wang et al² identify a first endothelial receptor for AGGF1. They used a targeted study of integrins to identify a receptor candidate and a series of informative deletion mutations to identify a 10-amino-acid region of AGGF1 to be its angiogenesis-inducing region. They definitively showed that the angiogenic properties of AGGF1 can be mediated through its interaction (via this small region) with the $\alpha 5 \beta 1$ integrin and downstream by activating Akt—the well-known serine/threonine-specific protein kinase.^{3,4} Thus, another endothelial cell receptor can be added to the long list of actors who, in whole or in part, play through Akt—a familiar intracellular signaling protein (Figure).

This commonality of endothelial cell receptors signaling through Akt raises the question of whether AGGF1 and its interaction with $\alpha 5 \beta 1$ is independent of VEGF (vascular endothelial growth factor) and the VEGF-VEGF receptor 2 pathway, perhaps the most extensively studied angiogenesis pathway that signals through Akt. In previous work,⁵ the authors showed that activation by AGGF1 did not alter the VEGF-VEGFR2 pathway, as measured by VEGFR2 levels and phosphorylation of one particular tyrosine on VEGFR2. While these

Correspondence to: Brian H. Annex, MD, Department of Medicine, Medical College of Georgia, Augusta University, 1120 15th St, BI 5076, Augusta, GA 30912. bannex@augusta.edu.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

Disclosures
None.

data are consistent with the two pathways being separate, this explanation is certainly not comprehensive as key factors such as subcellular localization of VEGFR2 can impact on the Akt response to receptor activation.⁶ When watching the scene from the outside of the cell (changes to ligand stimuli and cell surface receptors), the pathways may seem to be independent, but proof of independence is far more complicated, and this complexity increases further when considering Akt activation as the downstream node of convergence of what is easily an in excess of a dozen (known) ligand stimuli (Figure).

When considered from inside the cell, the scene is unclear and basic questions remain unanswered. If activated Akt is the signal (center of Figure) that is driving angiogenesis and vascular remodeling generally,^{3,4} does it matter what led to the activated Akt? Indeed, if not from the byproducts made in pathway activation, why should it matter? If generating a requisite amount of activated Akt (either total phosphorylated Akt [pAkt] or a threshold pAkt/Akt ratio) is needed for endothelial cell health, vascular development, or angiogenesis, why is it that VEGFR2 (or the dozen other receptors on endothelial cells) cannot act as the sole or compensatory pAkt generator? If the receptor generating the pAkt does not matter, then a testable hypothesis is that AGGF1 should work just as well when the VEGFR2 pathway is insufficient as it does when that critical pathway is intact. Considered purely from the perspective of Akt, any receptor leading to Akt could compensate for any other. Indeed, the authors showed in previous work⁵ that in endothelial cell culture, VEGF could activate Akt in low-AGGF1 conditions, and AGGF1 could (at least partially) activate Akt in siVEGFR2 conditions. However, the in vivo haploinsufficiency of VEGF and the partial haploinsufficiency of AGGF1 suggest that this compensation is likely not happening in vivo in development. Extending that consideration from 2 receptors generating Akt to 6 to 12 requires us to then explore whether these multiple upstream pathways are independent and, therefore, interchangeable and redundant, and perhaps cell, tissue, or pathology dependent based on the timing and location of ligand and receptor expression. Alternatively, are these pathways intersecting in ways that are not apparent yet, possibly through cross talk among the multiple pathways that they induce, and not solely Akt, which would provide an explanation for the observations of nonredundancy in heterozygous and homozygous knockouts. Ultimately this becomes a systems biology and computational problem that needs to be studied looking at multiple pathways simultaneously, to answer the question of why so many separate, seemingly independent receptor-mediated pathways in endothelial cells all function and act through the same pAkt node.

The authors have done an excellent job in identifying $\alpha 5\beta 1$ as a receptor for AGGF1 that activates Akt and the angiogenesis scene. However, showing that AGGF1 interaction with $\alpha 5\beta 1$ can activate Akt does not mean it does activate Akt across various developmental and physiological situations. To that end, it is worth noting that the article focused on the interaction of the AGGF1 with integrins and thus the authors provide evidence for an integrin-Akt pathway, but the study was not designed to (and did not) exclude other potential effects or interactions of AGGF1. Also consider, the half-maximal AGGF1 binding concentration is 18.7 nmol/L, meaning that concentrations similar to this or higher are likely needed to see significant activation of the pathway identified by the authors. In this article, they use 79 nmol/L AGGF1 to stimulate the response, but plasma levels of AGGF1 are more typically subnano-molar, so it is possible that this pathway can be activated in culture

but doesn't get activated in vivo. If it does, this suggests that either the ligand is highly upregulated or locally concentrated, perhaps more so in certain physiological or pathological situations. There is some precedent for this in the VEGF system, where the concentrations used to stimulate endothelial cell responses in culture are much higher than soluble VEGF levels that activate endothelial cells in vivo; in that case, at least part of the explanation for the difference rests on local concentrating of ligand by proteoglycans or extracellular matrix proteins,⁶ so it remains to be seen how AGGF1's low physiological concentrations might be potentiated to make them effective and relevant for angiogenesis in vivo.

Our understanding of the complex processes that regulate angiogenesis continue to evolve. Indeed much of that work focuses on ligand-receptor interactions and intracellular signals through Akt. This article adds one more receptor to the scene and further encourages us to look at what happens when so many pathways converge and Akt on the same stage.

Sources of Funding

This work is supported by R01HL150003 (B.H. Annex), R01HL1148590 (B.H. Annex), R01HL141325 (B.H. Annex), R01HL101200 (F. Mac Gabhann and B.H. Annex), and R01GM129074 (F. Mac Gabhann and B.H. Annex).

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Figure. Many cell surface receptors on endothelial cells are known to induce phosphorylated Akt (pAkt) following stimulation, including various receptor tyrosine kinases,⁷ GPCRs,⁸ and other integrins⁹ beyond the $\alpha\text{5}\beta\text{1}$ identified in this issue.²

It is not clear whether the total amount of pAkt or the relative amount (pAkt/Akt ratio) is the key driver of vascular development and remodeling, but pAkt is clearly a confluence of over a dozen ligand-receptor stimuli that induce or modulate vascular remodeling. Created with [BioRender.com](https://www.biorender.com/).