CANCER

Convergence of VEGF and YAP/TAZ signaling: Implications for angiogenesis and cancer biology

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Vascular endothelial growth factor (VEGF) stimulates endothelial cells to promote both developmental and pathological angiogenesis. VEGF also directly affects tumor cells and is associated with the initiation, progression, and recurrence of tumors, as well as the emergence and maintenance of cancer stem cells (CSCs). Studies have uncovered the importance of the transcriptional regulators YAP and TAZ in mediating VEGF signaling. For example, VEGF stimulates the GTPase activity of Rho family members and thereby alters cytoskeletal dynamics, which contributes to the activation of YAP and TAZ. In turn, YAP- and TAZ-mediated changes in gene expression sustain Rho family member activity and cytoskeletal effects to promote both vascular growth and remodeling in endothelial cells and the acquisition of stem-like traits in tumor cells. In this Review, we discuss how these findings further explain the pathophysiological roles of VEGF and YAP/TAZ, identify their connections to other receptor-mediated pathways, and reveal ways of therapeutically targeting their convergent signals in patients.

Introduction

Vascular endothelial growth factor (VEGF) was identified and isolated as an endothelial cell–specific mitogen that has the capacity to induce developmental and pathological angiogenesis (*1*, *2*). Since then, studies have revealed the ability of VEGF to directly target tumor cells, especially cells with stem-like traits referred to as cancer stem cells (CSCs), and consequently contribute to tumor initiation, progression, and recurrence (*3*). These vital functions of VEGF are mediated by receptors expressed on endothelial and tumor cells, including receptor tyrosine kinases [vascular endothelial growth factor receptor-1 (VEGFR1) and VEGFR2] and the neuropilins (NRPs), the latter of which function as VEGF co-receptors (*4*–*8*). The mechanisms by which these VEGFRs execute these diverse functions are of paramount importance for their potential as therapeutic targets. Not surprisingly, therefore, VEGF-mediated signaling has been studied intensely, but much remains to be learned; in particular, a better understanding of how VEGF signaling affects the cell biology that underlies vascular growth and remodeling (angiogenesis) and self-renewal (CSCs) is needed. In this direction, the convergence of VEGF and Hippo signaling has the potential to provide considerably new insight into angiogenesis and CSC function.

The Hippo pathway is critical for development because it restricts proliferation and controls organ size (*9*). Inhibition of this pathway results in the activation of YAP and TAZ, transcriptional coactivators that have profound effects on cell behavior. Active YAP and TAZ reside in the nucleus where they associate with the TEA domain (TEAD) family of transcription factors and regulate the expression of numerous targets collectively known as a "YAP/TAZ signature." Several cues, such as high cell density and polarity, can activate the core Hippo tumor suppressor pathway and thereby inhibit YAP/TAZ. This pathway consists of a kinase cascade mediated by the kinases MST1 and MST2 (MST1/2), which phosphorylate and activate the kinases LATS1 and LATS2 (LATS1/2) (*10*). Activated LATS1/2 directly phosphorylate YAP/TAZ at several conserved residues, with Ser^{127} of YAP and Ser^{89} of TAZ being the major and most heavily studied LATS1/2-mediated phosphorylation sites. YAP/TAZ phosphorylation creates binding sites for the protein 14-3-3, which results in their cytoplasmic retention, separation from the TEAD family of transcription factors, and functional inactivation (*10*, *11*). In addition to core MST/LATS kinase Hippo signaling, YAP/TAZ activity can be controlled by several other molecular factors termed the "extended" Hippo pathway. Although the core Hippo pathway and its extended components are well characterized (*12*), a rigorous understanding of signaling at the cell surface that leads to inactivation of the Hippo pathway and contributes to enhanced YAP/TAZ activity is still emerging. What has been shown recently in several studies is that VEGF signaling can promote YAP/TAZ activation in endothelial and tumor cells. A common theme that has emerged from these studies is that VEGF signaling affects the activity of Rho family guanosine triphosphatases (GTPases) and cytoskeletal dynamics, which contribute to YAP/TAZ activation, and that YAP/TAZ-mediated transcriptional changes sustain these effects to promote vascular growth and remodeling in endothelial cells and the acquisition of stem-like traits in tumor cells (Fig. 1). These findings add substantial insight both to the pathophysiological importance of VEGF and YAP/TAZ and to their connection to other receptor-mediated pathways. This Review discusses these findings and highlights areas for future study.

YAP/TAZ as Effectors of VEGF Signaling in Developmental Angiogenesis

The vascularization of tissues during development is a precisely orchestrated angiogenic process mediated primarily by VEGF that involves profound changes in endothelial cells including proliferation, sprouting, directed migration, and reorganization of cell-cell junctions (*13*). These diverse functions of endothelial cells are mediated, in part, by transcriptional and cytoskeletal alterations, but the mechanisms responsible for these alterations are still being elucidated. For this reason, recent reports describing VEGF-mediated YAP/TAZ activation in promoting developmental angiogenesis are a substantial advance. One study observed VEGF-mediated YAP/TAZ activation in cultured endothelial cells and, through endothelial

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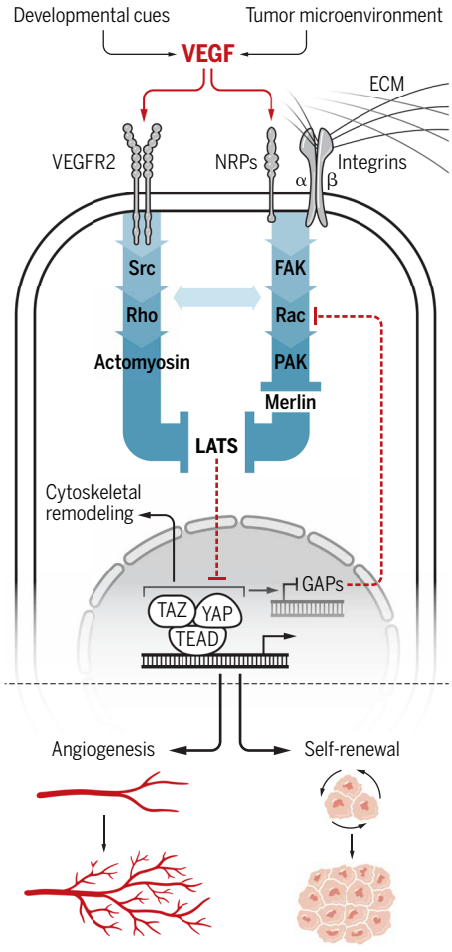

Fig. 1. Schematic of VEGF and YAP/TAZ signaling thus far described in endothelial and tumor cells. Emerging studies suggest that VEGF signaling mediated by VEGFR2 and/or NRPs increases the activity of Rho family GTPases, resulting in LATS inhibition and YAP/TAZ activation, with consequent promotion of angiogenesis and stem cell maintenance. Integrins have a key role in this signaling by associating with NRPs and engaging the extracellular matrix (ECM). VEGF signaling can involve Src-mediated Rho activation and the consequent activation of YAP/TAZ through cytoskeletal dynamics. It can also inhibit LATS by a Rac1-dependent mechanism that involves p21-activated kinase (PAK)–mediated inhibition of Merlin. An important question is whether these two mechanisms function in concert to promote YAP/TAZ activation in response to VEGF signaling. YAP/TAZ-mediated transcription can alter the expression of genes involved in cytoskeletal remodeling and in Rho and Rac1 activation, such as through the transcriptional repression of Rac GTPase activating proteins (GAPs) that normally turn off Rac, thereby establishing a positive feedback loop.

cell–specific deletions of YAP/TAZ in mice, demonstrated their importance in embryonic and postnatal vascular development: YAP/TAZ deletion resulted in an impaired vascular response to VEGF, indicating that YAP/TAZ are important regulators of angiogenesis downstream of VEGF (*14*). The mechanism by which VEGF activates YAP/TAZ involves VEGFR2 activation of Src family kinases and subsequent cytoskeletal rearrangements mediated by Src activation of Rho family GTPases. Inhibiting this VEGF-VEGFR2-Src-RhoGTPase mechanism promoted LATS-mediated phosphorylation (and hence inhibition) of YAP/TAZ. This finding is consistent with reports that activation of Rho GTPase by upstream G protein (heterotrimeric guanine nucleotide–binding protein)–coupled receptor signaling and consequent cytoskeletal alterations activate YAP/TAZ (*15*–*17*), although there is evidence that this regulation of YAP/TAZ can occur independently of LATS kinases (*15*). Also described was a positive feedback loop enabled by YAP/TAZ-dependent transcriptional changes that sustained cytoskeletal alterations and Rho GTPase activity; specifically, chromatin immunoprecipitation sequencing analysis revealed that the VEGF-VEGFR2-Src-RhoGTPase signaling axis promotes YAP/TAZ-dependent expression of several cytoskeletal remodeling genes, including the gene encoding myosin 1C (*14*). Myosin 1C is implicated in trafficking VEGFR2 from the Golgi apparatus to the plasma membrane (*18*). Consequently, enhanced YAP/TAZ activity was shown to be critical to retaining VEGFR2 on the cell surface and promoting a feedforward loop sustaining VEGF-VEGFR2-Src-RhoGTPase–mediated activation of YAP/TAZ that contributes to developmental angiogenesis (*14*).

Another interesting study concluding that VEGF activates YAP/TAZ in endothelial cells focused more on the cell biology of how YAP/TAZ influence angiogenesis (*19*). Analysis of mice with endothelial cell–specific deletion of YAP/TAZ revealed defects in sprouting at the vascular front where tip endothelial cells are exposed to VEGF, which was associated with defective formation of filopodia and lumen and with a reduction and disarrangement of endothelial cell junctions. This phenotype was attributed to the ability of YAP/TAZ to modulate the actomyosin cytoskeleton in response to VEGF by sustaining activation of the Rho family GTPase CDC42. Although the mechanism by which YAP/TAZ contribute to CDC42 activation was not addressed, it was presumed to involve transcriptionally competent YAP/TAZ in the nucleus. This point is notable because another study, which did not consider VEGF signaling, concluded that cytoplasmic YAP regulates CDC42 in the migration of tip endothelial cells (*20*). This observation is somewhat surprising, but a previous study not involving VEGF signaling found that cytoplasmic YAP influences cytokinesis through its regulation of Rho GTPases and Rho GTPase regulatory proteins (*21*). It will be important to assess whether VEGF signaling affects cytoplasmic YAP and to understand its role in angiogenesis. A broader issue is the role of nuclear versus cytoplasmic YAP in the regulation of Rho GTPases and their regulation by both transcriptional and nontranscriptional mechanisms. In addition, it is likely that YAP and TAZ differ in this regard. Although both YAP and TAZ are prone to degradation in the cytoplasm, cytoplasmic TAZ may not have a nontranscriptional function because it has a shorter half-life than YAP and is highly unstable in the cytoplasm (*9*, *22*, *23*).

Other recent studies have used different approaches to arrive at the conclusion that YAP/TAZ are key effectors of VEGF-mediated angiogenesis. The use of a novel bioluminescence-based biosensor that monitors LATS kinase activity in combination with a library of small-molecule kinase inhibitors revealed that VEGFR signaling inhibits LATS activity and, consequently, promotes YAP/TAZ activation in endothelial cells (*24*). The mechanism identified involves VEGFR-mediated phosphatidylinositol 3-kinase (PI3K)/mitogen-activated protein kinase (MAPK) activation that suppresses activation of MST1/2, which impedes their ability to activate the LATS kinases. On the basis of this information, this study also demonstrated that YAP/TAZ are critical for VEGF-induced angiogenesis in vivo. They also found that YAP/TAZ control the expression of the genes encoding angiopoietin-2 and CYR61 (cysteine-rich angiogenic

inducer 61), proteins that—as their names imply—are involved in angiogenesis (*25*). Another study observed that VEGF stimulation of endothelial cells reduced the phosphorylation of YAP and increased its nuclear localization, hallmarks of YAP activation (*26*). This result prompted the generation of transgenic mice (Tie2Cre-YAP^{Tg}) in which YAP was overexpressed in endothelial cells, which resulted in increased retinal angiogenesis. Notably, YAP was shown to partner with signal transducer and activator of transcription 3 (STAT3) and promote nuclear STAT3 localization, and evidence that the YAP/STAT3 complex enhances angiogenic functions in culture was provided. One mechanism proposed is that YAP increases the STAT3-mediated transcription of the gene encoding angiopoietin-2, which is related to the conclusion of the previous study (*14*).

The authors of the studies demonstrating that VEGF signaling activates YAP/TAZ in endothelial cells have proposed different mediating mechanisms (Src-Rho family GTPase, PI3K/MAPK, and STAT3). Future work should focus on whether these pathways can be integrated into a unified mechanism for VEGF-VEGFR–mediated YAP/TAZ activation in endothelial cells. The possibility that a central tenet of this mechanism involves VEGF-mediated activation of Rho family GTPases and the consequent impact on cytoskeletal dynamics and mechanical tension seems likely, especially given the existing literature on YAP/TAZ activation by mechanical forces (*27*). Nonetheless, it is premature to exclude other modes of YAP/TAZ activation. It is also important to note that other studies had implicated Hippo signaling in angiogenesis (*28*), but they did not assess the potential role of VEGF signaling in regulating this pathway. This issue is timely because a recent study implicated bone morphogenetic protein signaling and discounted the involvement of VEGF in YAP/TAZ-mediated regulation of sprouting angiogenesis in the mouse retina (*29*). Clearly, more work is needed to define the contribution of VEGF signaling to YAP/TAZ activation in developmental angiogenesis and to assess its relationship to other signaling pathways. The possibility that YAP/TAZ can be activated in endothelial cells by blood flow independently of VEGF and Hippo signaling also needs to be considered (*30*).

Role of VEGF-Mediated YAP/TAZ Activation in Cancer Cells

The seminal finding that VEGF-mediated angiogenesis is a hallmark of cancer sparked intense interest in the identification of signaling pathways in tumor-associated endothelial cells that are driven by VEGF and how they can be exploited for therapeutic purposes (*31*). The importance of VEGF in cancer has been amplified by the realization that VEGF signaling is critical for the function of some cancer cells, especially CSCs. CSCs are defined as a subpopulation of cells that exhibit properties of stem cells, including self-renewal and function in tumor initiation, differentiation into heterogeneous cancer cell lineages, and therapy resistance (*32*). Autocrine VEGF signaling has emerged as an essential pathway for sustaining self-renewal and other CSC functions in several types of cancer (*3*). Many, but not all, of these studies have highlighted a key role for the NRPs (NRP1 and NRP2) in mediating this signaling and discounted the contribution of VEGFRs (*33*–*36*). This latter observation has notable implications for therapy because the most common anti-VEGF drug (bevacizumab) blocks the binding of VEGF to receptor tyrosine kinases but not to NRPs (*37*). This observation may explain, in part, the dismal efficacy of bevacizumab for many cancers (*38*, *39*), and it highlights the potential benefit of directly targeting the NRPs as an approach to inhibiting VEGF signaling in CSCs. For this reason, understanding how VEGF-NRP signaling affects CSCs is a timely and clinically important question.

There is now compelling evidence that YAP/TAZ are essential for the function of CSCs and other aspects of aggressive tumor behavior (*40*). This finding raises the important issue of how YAP/TAZ activity is regulated to affect CSCs and the behavior of aggressive cancers. Presumably, this regulation occurs by factors and conditions in the tumor microenvironment, but not much is known about the nature of these signals. There is evidence, for example, that the matrix protein laminin 511 is a component of a CSC niche and that the engagement of this laminin with a splice variant of the α6β1 integrin (α6Bβ1) sustains TAZ activity in breast CSCs (*41*).

The recent report that autocrine VEGF signaling through NRP2 promotes the self-renewal of breast CSCs by sustaining TAZ activation links a key component of the tumor microenvironment (VEGF) with TAZ in CSC function (*42*). This study highlighted a critical role of Rac1 in TAZ activation by VEGF-NRP2 signaling, which is consistent with the key role for Rho family GTPases in promoting YAP/TAZ activation observed in endothelial cells (*19*, *26*). One mechanism proposed by which Rac1 contributes to TAZ activation involves PAK (a Rac-activated kinase) that phosphorylates Merlin, the protein encoded by the *NF2* (neurofibromatosis type 2) gene, on Ser^{518} (*43*–*45*), which inhibits the phosphorylation of LATS (*12*, *46*–*48*) and, consequently, facilitates the activation of YAP/TAZ (*42*, *49*). An essential component of this mechanism was the repression of the expression of the Rac GAP β2-chimaerin by TAZ. That repression of β2-chimaerin enabled the activation of Rac1 and sustained TAZ activation, thereby forming a positive feedback loop downstream of the VEGF-NRP2 signal. This study also found that a TAZ/TEAD complex binds to the promoter region of the gene encoding β2-chimaerin and contributes to its repression. Another study in gastric cancer, which did not involve VEGF signaling, found that the expression of the Rho GAP ARHGAP29 is controlled by YAP, which modulates Rho signaling to promote a metastatic phenotype (*50*). These observations reveal the ability of YAP/TAZ-dependent transcription to affect CSCs and promote metastasis by regulating the expression of Rho family GAPs.

Interestingly, the available data, albeit limited, indicate that VEGF-mediated YAP/TAZ activation in CSCs can be mediated by NRPs independently of VEGFRs (*42*, *51*). This conclusion is substantiated by a study on prostate cancer cells, which found that VEGF-NRP–mediated Rac1 activation is VEGFR independent (*52*). As alluded to above, although there is evidence that VEGF signaling in CSCs can be mediated by VEGFRs, notably, those studies did not involve an assessment of YAP/TAZ activation (*53*–*55*). Given that NRPs function as co-receptors and lack intrinsic signaling properties (*56*–*58*), the question of how NRPs activate YAP/TAZ arises. One mechanism may involve the ability of NRP2 to function as a co-receptor for the α6β1 integrin. This integrin associates with NRP2 in breast cancer cells, and this interaction facilitates the signaling potential of this integrin, including its ability to activate focal adhesion kinase (FAK) (*34*, *59*), which is consistent with the report that VEGF-NRP2 activates Rac1 in a FAK-dependent manner (*42*). This finding is consistent with the observation that NRP2 and the α6β1 integrin are markers of breast CSCs (*34*, *60*). Another, more recent, study confirmed both the importance of NRPs in regulating

α6 integrin signaling in epidermal CSCs and the lack of VEGFR involvement (*51*). This study also found that VEGF-NRP signaling activates YAP by FAK/Src-mediated inhibition of LATS phosphorylation. In contrast to the previous report on the α6β1 integrin, however, this study argued that the α6β4 integrin interacts with NRP1 on the surface of CSCs and that this interaction promotes YAP activation. This discrepancy may reflect differences in α6 integrin expression between breast and epidermal CSCs, but these studies nonetheless demonstrate that NRPs can promote YAP/TAZ activation without VEGFR involvement by co-opting integrin signaling. More work is needed, however, to definitively exclude the involvement of VEGFRs in VEGF-mediated YAP/TAZ activation, especially in light of their key role in YAP/TAZ activation in endothelial cells and other reports on cancer cells.

Future Exploration of Convergent VEGF- and YAP/TAZ-Dependent Mechanisms

The role of YAP/TAZ activation in executing the functional consequences of VEGF signaling in endothelial and CSCs that has become apparent is providing new insight into the mechanisms that underlie angiogenesis and the acquisition of stem-like traits. A central theme that has emerged from these studies is the critical role of the Rho family of small GTPases in mediating the signaling events initiated by VEGF to activate YAP/TAZ. These GTPases contribute to YAP/TAZ activation indirectly by altering cytoskeletal dynamics and directly by inhibiting LATS phosphorylation. The transcriptional alterations that result from YAP/TAZ activation can initiate a positive feedback loop that sustains Rho GTPase activation. Although aspects of this signaling network had been established previously, the novelty of the recent studies highlighted in this Review is the ability of VEGF and VEGFRs to orchestrate this network. This mode of YAP/TAZ regulation is significant because VEGF signaling itself is tightly regulated during development and aberrantly activated by the tumor microenvironment, which provides a pathophysiological context for YAP/TAZ activation. Looking forward, a better understanding of how the different types of VEGFRs (receptor tyrosine kinases and NRPs) contribute to YAP/TAZ activation and their interaction with other surface receptors is needed. These studies should consider the role of mechanical forces imposed by the ECM and tissue microenvironment in VEGFR-mediated YAP/TAZ activation and should attempt to delineate the specific contributions of different Rho GTPase family members to YAP/TAZ activation in the context of VEGF signaling (Fig. 1). However, much remains to be learned both about how YAP and TAZ mediate gene expression changes to execute the VEGF-induced effects in endothelial and tumor cells and about how YAP and TAZ may differ in this regard. The possibility that cytoplasmic YAP is regulated by VEGF signaling and contributes to angiogenesis and CSC function should also be considered (Fig. 1). The impact of this work is likely to be substantial, given the intense interest in targeting VEGF signaling as a therapeutic approach to inhibiting angiogenesis and CSC growth and survival in cancer patients.

REFERENCES AND NOTES

1. D. W. Leung, G. Cachianes, W. J. Kuang, D. V. Goeddel, N. Ferrara, Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* **246**, 1306–1309 (1989).
2. D. R. Senger, S. J. Galli, A. M. Dvorak, C. A. Perruzzi, V. S. Harvey, H. F. Dvorak, Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science* **219**, 983–985 (1983).
3. H. L. Goel, A. M. Mercurio, VEGF targets the tumour cell. *Nat. Rev. Cancer* **13**, 871–882 (2013).
4. H. L. Goel, A. M. Mercurio, Enhancing integrin function by VEGF/neuropilin signaling: Implications for tumor biology. *Cell Adh. Migr.* **6**, 554–560 (2012).
5. G. Neufeld, O. Kessler, Y. Herzog, The interaction of Neuropilin-1 and Neuropilin-2 with tyrosine-kinase receptors for VEGF. *Adv. Exp. Med. Biol.* **515**, 81–90 (2002).
6. S. Soker, S. Takashima, H. Q. Miao, G. Neufeld, M. Klagsbrun, Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. *Cell* **92**, 735–745 (1998).
7. E. Sulpice, J. Plouët, M. Bergé, D. Allanic, G. Tobelem, T. Merkulova-Rainon, Neuropilin-1 and neuropilin-2 act as coreceptors, potentiating proangiogenic activity. *Blood* **111**, 2036–2045 (2008).
8. S. Koch, L. A. van Meeteren, E. Morin, C. Testini, S. Weström, H. Björkelund, S. Le Jan, J. Adler, P. Berger, L. Claesson-Welsh, NRP1 presented in *trans* to the endothelium arrests VEGFR2 endocytosis, preventing angiogenic signaling and tumor initiation. *Dev. Cell* **28**, 633–646 (2014).
9. S. Piccolo, S. Dupont, M. Cordenonsi, The biology of YAP/TAZ: Hippo signaling and beyond. *Physiol. Rev.* **94**, 1287–1312 (2014).
10. Z. Meng, T. Moroishi, K.-L. Guan, Mechanisms of Hippo pathway regulation. *Genes Dev.* **30**, 1–17 (2016).
11. F. Kanai, P. A. Marignani, D. Sarbassova, R. Yagi, R. A. Hall, M. Donowitz, A. Hisaminato, T. Fujiwara, Y. Ito, L. C. Cantley, M. B. Yaffe, TAZ: A novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins. *EMBO J.* **19**, 6778–6791 (2000).
12. S. W. Plouffe, Z. Meng, K. C. Lin, B. Lin, A. W. Hong, J. V. Chun, K.-L. Guan, Characterization of Hippo pathway components by gene inactivation. *Mol. Cell* **64**, 993–1008 (2016).
13. J. J. Haigh, Role of VEGF in organogenesis. *Organogenesis* **4**, 247–256 (2008).
14. X. Wang, A. Freire Valls, G. Schermann, Y. Shen, I. M. Moya, L. Castro, S. Urban, G. M. Solecki, F. Winkler, L. Riedemann, R. K. Jain, M. Mazzone, T. Schmidt, T. Fischer, G. Halder, C. Ruiz de Almodóvar, YAP/TAZ orchestrate VEGF signaling during developmental angiogenesis. *Dev. Cell* **42**, 462–478.e7 (2017).
15. X. Feng, M. S. Degese, R. Iglesias-Bartolome, J. P. Vaque, A. A. Molinolo, M. Rodrigues, M. R. Zaidi, B. R. Ksander, G. Merlino, A. Sodhi, Q. Chen, J. S. Gutkind, Hippo-independent activation of YAP by the GNAQ uveal melanoma oncogene through a trio-regulated rho GTPase signaling circuitry. *Cancer Cell* **25**, 831–845 (2014).
16. H. W. Park, Y. C. Kim, B. Yu, T. Moroishi, J.-S. Mo, S. W. Plouffe, Z. Meng, K. C. Lin, F.-X. Yu, C. M. Alexander, C. Wang, K.-L. Guan, Alternative Wnt signaling activates YAP/TAZ. *Cell* **162**, 780–794 (2015).
17. F.-X. Yu, B. Zhao, N. Panupinthu, J. L. Jewell, I. Lian, L. H. Wang, J. Zhao, H. Yuan, K. Tumaneng, H. Li, X.-D. Fu, G. B. Mills, K.-L. Guan, Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling. *Cell* **150**, 780–791 (2012).
18. A. Tiwari, J.-J. Jung, S. M. Inamdar, D. Nihalani, A. Choudhury, The myosin motor Myo1c is required for VEGFR2 delivery to the cell surface and for angiogenic signaling. *Am. J. Physiol. Heart Circ. Physiol.* **304**, H687–H696 (2013).
19. J. Kim, Y. H. Kim, J. Kim, D. Y. Park, H. Bae, D.-H. Lee, K. H. Kim, S. P. Hong, S. P. Jang, Y. Kubota, Y.-G. Kwon, D.-S. Lim, G. Y. Koh, YAP/TAZ regulates sprouting angiogenesis and vascular barrier maturation. *J. Clin. Invest.* **127**, 3441–3461 (2017).
20. M. Sakabe, J. Fan, Y. Odaka, N. Liu, A. Hassan, X. Duan, P. Stump, L. Byerly, M. Donaldson, J. Hao, M. Fruttiger, Q. R. Lu, Y. Zheng, R. A. Lang, M. Xin, YAP/TAZ-CDC42 signaling regulates vascular tip cell migration. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 10918–10923 (2017).
21. D. A. Bui, W. Lee, A. E. White, J. W. Harper, R. C. J. Schackmann, M. Overholtzer, L. M. Selfors, J. S. Brugge, Cytokinesis involves a nontranscriptional function of the Hippo pathway effector YAP. *Sci. Signal.* **9**, ra23 (2016).
22. C.-Y. Liu, Z.-Y. Zha, X. Zhou, H. Zhang, W. Huang, D. Zhao, T. Li, S. W. Chan, C. J. Lim, W. Hong, S. Zhao, Y. Xiong, Q.-Y. Lei, K.-L. Guan, The hippo tumor pathway promotes TAZ degradation by phosphorylating a phosphodegron and recruiting the $SCF^{\beta\text{-}TrCP}$ E3 ligase. *J. Biol. Chem.* **285**, 37159–37169 (2010).
23. W. Huang, X. Lv, C. Liu, Z. Zha, H. Zhang, Y. Jiang, Y. Xiong, Q.-Y. Lei, K. L. Guan, The N-terminal phosphodegron targets TAZ/WWTR1 protein for $SCF^{\beta\text{-}TrCP}$-dependent degradation in response to phosphatidylinositol 3-kinase inhibition. *J. Biol. Chem.* **287**, 26245–26253 (2012).
24. T. Azad, H. J. Janse van Rensburg, E. D. Lightbody, B. Neveu, A. Champagne, A. Ghaffari, V. R. Kay, Y. Hao, H. Shen, B. Yeung, B. A. Croy, K. L. Guan, F. Pouliot, J. Zhang, C. J. B. Nicol, X. Yang, A LATS biosensor screen identifies VEGFR as a regulator of the Hippo pathway in angiogenesis. *Nat. Commun.* **9**, 1061 (2018).
25. H.-J. Choi, H. Zhang, H. Park, K.-S. Choi, H.-W. Lee, V. Agrawal, Y.-M. Kim, Y.-G. Kwon, Yes-associated protein regulates endothelial cell contact-mediated expression of angiopoietin-2. *Nat. Commun.* **6**, 6943 (2015).
26. J. He, Q. Bao, Y. Zhang, M. Liu, H. Lv, Y. Liu, L. Yao, B. Li, C. Zhang, S. He, G. Zhai, Y. Zhu, X. Liu, K. Zhang, X.-J. Wang, M.-H. Zou, Y. Zhu, D. Ai, Yes-associated protein promotes angiogenesis via signal transducer and activator of transcription 3 in endothelial cells. *Circ. Res.* **122**, 591–605 (2018).

27. A. Totaro, T. Panciera, S. Piccolo, YAP/TAZ upstream signals and downstream responses. *Nat. Cell Biol.* **20**, 888–899 (2018).
28. J. A. Park, Y.-G. Kwon, Hippo-YAP/TAZ signaling in angiogenesis. *BMB Rep.* **51**, 157–162 (2018).
29. F. Neto, A. Klaus-Bergmann, Y. T. Ong, S. Alt, A.-C. Vion, A. Szymborska, J. R. Carvalho, I. Hollfinger, E. Bartels-Klein, C. A. Franco, M. Potente, H. Gerhardt, YAP and TAZ regulate adherens junction dynamics and endothelial cell distribution during vascular development. *eLife* **7**, e31037 (2018).
30. H. Nakajima, K. Yamamoto, S. Agarwala, K. Terai, H. Fukui, S. Fukuhara, K. Ando, T. Miyazaki, Y. Yokota, E. Schmelzer, H.-G. Belting, M. Affolter, V. Lecaudey, N. Mochizuki, Flow-dependent endothelial YAP regulation contributes to vessel maintenance. *Dev. Cell* **40**, 523–536.e6 (2017).
31. N. Ferrara, VEGF as a therapeutic target in cancer. *Oncology* **69** (suppl. 3), 11–16 (2005).
32. E. Batlle, H. Clevers, Cancer stem cells revisited. *Nat. Med.* **23**, 1124–1134 (2017).
33. B. Beck, G. Driessens, S. Goossens, K. K. Youssef, A. Kuchnio, A. Caauwe, P. A. Sotiropoulou, S. Loges, G. Lapouge, A. Candi, G. Mascre, B. Drogat, S. Dekoninck, J. J. Haigh, P. Carmeliet, C. Blanpain, A vascular niche and a VEGF-Nrp1 loop regulate the initiation and stemness of skin tumours. *Nature* **478**, 399–403 (2011).
34. H. L. Goel, B. Pursell, C. Chang, L. M. Shaw, J. Mao, K. Simin, P. Kumar, C. W. Vander Kooi, L. D. Shultz, D. L. Greiner, J. H. Norum, R. Toftgard, C. Kuperwasser, A. M. Mercurio, GLI1 regulates a novel neuropilin-2/α6β1 integrin based autocrine pathway that contributes to breast cancer initiation. *EMBO Mol. Med.* **5**, 488–508 (2013).
35. J. M. Siegle, A. Basin, A. Sastre-Perona, Y. Yonekubo, J. Brown, R. Sennett, M. Rendl, A. Tsirigos, J. A. Carucci, M. Schober, SOX2 is a cancer-specific regulator of tumour initiating potential in cutaneous squamous cell carcinoma. *Nat. Commun.* **5**, 4511 (2014).
36. M. Snuderl, A. Batista, N. D. Kirkpatrick, C. Ruiz de Almodovar, L. Riedemann, E. C. Walsh, R. Anolik, Y. Huang, J. D. Martin, W. Kamoun, E. Knevels, T. Schmidt, C. T. Farrar, B. J. Vakoc, N. Mohan, E. Chung, S. Roberge, T. Peterson, C. Bais, B. H. Zhelyazkova, S. Yip, M. Hasselblatt, C. Rossig, E. Niemeyer, N. Ferrara, M. Klagsbrun, D. G. Duda, D. Fukumura, L. Xu, P. Carmeliet, R. K. Jain, Targeting placental growth factor/neuropilin 1 pathway inhibits growth and spread of medulloblastoma. *Cell* **152**, 1065–1076 (2013).
37. E. Geretti, L. A. van Meeteren, A. Shimizu, A. C. Dudley, L. Claesson-Welsh, M. Klagsbrun, A mutated soluble neuropilin-2 B domain antagonizes vascular endothelial growth factor bioactivity and inhibits tumor progression. *Mol. Cancer Res.* **8**, 1063–1073 (2010).
38. G. Bergers, D. Hanahan, Modes of resistance to anti-angiogenic therapy. *Nat. Rev. Cancer* **8**, 592–603 (2008).
39. D. Lambrechts, H.-J. Lenz, S. de Haas, P. Carmeliet, S. J. Scherer, Markers of response for the antiangiogenic agent bevacizumab. *J. Clin. Oncol.* **31**, 1219–1230 (2013).
40. F. Zanconato, M. Cordenonsi, S. Piccolo, YAP/TAZ at the roots of cancer. *Cancer Cell* **29**, 783–803 (2016).
41. C. Chang, H. L. Goel, H. Gao, B. Pursell, L. D. Shultz, D. L. Greiner, S. Ingerpuu, M. Patarroyo, S. Cao, E. Lim, J. Mao, K. K. McKee, P. D. Yurchenco, A. M. Mercurio, A laminin 511 matrix is regulated by TAZ and functions as the ligand for the α6Bβ1 integrin to sustain breast cancer stem cells. *Genes Dev.* **29**, 1–6 (2015).
42. A. L. Elaimy, S. Guru, C. Chang, J. Ou, J. J. Amante, L. J. Zhu, H. L. Goel, A. M. Mercurio, VEGF–neuropilin-2 signaling promotes stem-like traits in breast cancer cells by TAZ-mediated repression of the Rac GAP β2-chimaerin. *Sci. Signal.* **11**, eaao6897 (2018).
43. J. L. Kissil, K. C. Johnson, M. S. Eckman, T. Jacks, Merlin phosphorylation by p21-activated kinase 2 and effects of phosphorylation on merlin localization. *J. Biol. Chem.* **277**, 10394–10399 (2002).
44. R. J. Shaw, J. G. Paez, M. Curto, A. Yaktine, W. M. Pruitt, I. Saotome, J. P. O'Bryan, V. Gupta, N. Ratner, C. J. Der, T. Jacks, A. I. McClatchey, The *Nf2* tumor suppressor, merlin, functions in Rac-dependent signaling. *Dev. Cell* **1**, 63–72 (2001).
45. G.-H. Xiao, A. Beeser, J. Chernoff, J. R. Testa, p21-activated kinase links Rac/Cdc42 signaling to merlin. *J. Biol. Chem.* **277**, 883–886 (2002).
46. W. Li, J. Cooper, L. Zhou, C. Yang, H. Erdjument-Bromage, D. Zagzag, M. Snuderl, M. Ladanyi, C. O. Hanemann, P. Zhou, M. A. Karajannis, F. G. Giancotti, Merlin/NF2 loss-driven tumorigenesis linked to $CRL4^{DCAF1}$-mediated inhibition of the Hippo pathway kinases Lats1 and 2 in the nucleus. *Cancer Cell* **26**, 48–60 (2014).
47. W. Li, L. You, J. Cooper, G. Schiavon, A. Pepe-Caprio, L. Zhou, R. Ishii, M. Giovannini, C. O. Hanemann, S. B. Long, H. Erdjument-Bromage, P. Zhou, P. Tempst, F. G. Giancotti, Merlin/*NF2* suppresses tumorigenesis by inhibiting the E3 ubiquitin ligase $CRL4^{DCAF1}$ in the nucleus. *Cell* **140**, 477–490 (2010).
48. F. Yin, J. Yu, Y. Zheng, Q. Chen, N. Zhang, D. Pan, Spatial organization of Hippo signaling at the plasma membrane mediated by the tumor suppressor Merlin/NF2. *Cell* **154**, 1342–1355 (2013).
49. H. Sabra, M. Brunner, V. Mandati, B. Wehrle-Haller, D. Lallemand, A.-S. Ribba, G. Chevalier, P. Guardiola, M. R. Block, D. Bouvard, β1 integrin-dependent Rac/group I PAK signaling mediates YAP activation of Yes-associated protein 1 (YAP1) via NF2/merlin. *J. Biol. Chem.* **292**, 19179–19197 (2017).
50. Y. Qiao, J. Chen, Y. B. Lim, M. L. Finch-Edmondson, V. P. Seshachalam, L. Qin, T. Jiang, B. C. Low, H. Singh, C. T. Lim, M. Sudol, YAP regulates actin dynamics through ARHGAP29 and promotes metastasis. *Cell Rep.* **19**, 1495–1502 (2017).
51. D. Grun, G. Adhikary, R. L. Eckert, NRP-1 interacts with GIPC1 and α6/β4-integrins to increase YAP1/ΔNp63α-dependent epidermal cancer stem cell survival. *Oncogene* **37**, 4711–4722 (2018).
52. H. L. Goel, B. Pursell, L. D. Shultz, D. L. Greiner, R. A. Brekken, C. W. Vander Kooi, A. M. Mercurio, P-Rex1 promotes resistance to VEGF/VEGFR-targeted therapy in prostate cancer. *Cell Rep.* **14**, 2193–2208 (2016).
53. K. Liu, M. Hao, Y. Ouyang, J. Zheng, D. Chen, $CD133^+$ cancer stem cells promoted by VEGF accelerate the recurrence of hepatocellular carcinoma. *Sci. Rep.* **7**, 41499 (2017).
54. P. Hamerlik, J. D. Lathia, R. Rasmussen, Q. Wu, J. Bartkova, M. Lee, P. Moudry, J. Bartek Jr., W. Fischer, J. Lukas, J. N. Rich, J. Bartek, Autocrine VEGF–VEGFR2–Neuropilin-1 signaling promotes glioma stem-like cell viability and tumor growth. *J. Exp. Med.* **209**, 507–520 (2012).
55. D. Zhao, C. Pan, J. Sun, C. Gilbert, K. Drews-Elger, D. J. Azzam, M. Picon-Ruiz, M. Kim, W. Ullmer, D. El-Ashry, C. J. Creighton, J. M. Slingerland, VEGF drives cancer-initiating stem cells through VEGFR-2/Stat3 signaling to upregulate Myc and Sox2. *Oncogene* **34**, 3107–3119 (2015).
56. M. W. Parker, H.-F. Guo, X. Li, A. D. Linkugel, C. W. Vander Kooi, Function of members of the neuropilin family as essential pleiotropic cell surface receptors. *Biochemistry* **51**, 9437–9446 (2012).
57. E. Geretti, A. Shimizu, M. Klagsbrun, Neuropilin structure governs VEGF and semaphorin binding and regulates angiogenesis. *Angiogenesis* **11**, 31–39 (2008).
58. M. W. Parker, P. Xu, X. Li, C. W. Vander Kooi, Structural basis for selective vascular endothelial growth factor-A (VEGF-A) binding to neuropilin-1. *J. Biol. Chem.* **287**, 11082–11089 (2012).
59. H. L. Goel, B. Pursell, C. Standley, K. Fogarty, A. M. Mercurio, Neuropilin-2 regulates α6/β1 integrin in the formation of focal adhesions and signaling. *J. Cell Sci.* **125**, 497–506 (2012).
60. H. L. Goel, T. Gritsko, B. Pursell, C. Chang, L. D. Shultz, D. L. Greiner, J. H. Norum, R. Toftgard, L. M. Shaw, A. M. Mercurio, Regulated splicing of the α6 integrin cytoplasmic domain determines the fate of breast cancer stem cells. *Cell Rep.* **7**, 747–761 (2014).

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