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## Ninein Leads the Way in Vessel Sprouting

Victoria L. Bautch

Angiogenic sprouting is required to form an interconnected network of blood vessels. Vessel sprouting is essentially the coordinated migration of a group of endothelial cells toward a signal source, such as VEGF. This group of cells needs a leader, and much recent work has focused on how the leader, or “tip cell,” is chosen. However, how the tip cell reorganizes its cellular machinery to specialize in forward movement is not well understood. In this issue, Matsumoto and colleagues have identified ninein as a microtubule-binding protein with a dynamic cellular localization in endothelial cells that correlates with the behavior of the cell.<sup>1</sup> They have also elucidated a role for ninein in vessel tube formation in several models, thus providing the first evidence that ninein function is required for blood vessel formation. These findings help us begin to understand how extrinsic signals might link to cell behaviors in blood vessels, and they also have potential implications for cancer therapeutics, as discussed below.

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Ninein was identified as a centrosomal protein by Morgensen et al.,<sup>2</sup> and it can both nucleate and anchor microtubules at their minus-end. This suggests a complex relationship between ninein and microtubule (MT) dynamics in the cell, and in fact ninein is associated with anchoring MTs at the centrosome. Ninein is also able to anchor MTs at noncentrosomal sites, and this function is thought to be important when epithelial cells polarize their MT network away from the centrosome.<sup>2,3</sup> Ninein also associates with, and likely nucleates, short MTs (and perhaps non-MT intermediate filaments) in migrating cells, and this activity appears essential for proper migration.<sup>4</sup> Both gain- and loss-of-function in cells perturbs cellular processes and migration.<sup>4,5</sup> However, to date all reported studies were done in cultured cells.

Matsumoto et al were interested in a group of proteins that are tyrosine phosphorylated as endothelial cells undergo tube formation, reasoning that these proteins might have a critical role in tubulogenesis. Thus they performed a screen that yielded ninein as a major protein that was phosphorylated as endothelial cells formed tubes in culture. They went on to show that ninein was localized to both the centrosome and to noncentrosomal locations in endothelial cells, whereas nonendothelial cells had largely

a unique centrosomal location of ninein. This suggested that ninein function in endothelial cells is dynamic and important, and inspection of vessels in human tissues showed strong cytoplasmic localization of ninein that was enhanced in tumor vessels. Indeed, knockdown of ninein by siRNA significantly blocked both endothelial proliferation and tube formation, and because tube formation in this context did not require proliferation these were considered to be independent events. Matsumoto et al also used a novel technology called proximity ligation, which uses oligonucleotide-ligated secondary antibodies, and when in close proximity this configuration allows an in situ rolling circle DNA amplification reaction to occur. This detection method showed that ninein was tyrosine phosphorylated at its cytoplasmic location, and this phosphorylation was increased by incubation with VEGF. Finally, they used a model of vascular development in embryoid bodies to show that ninein localization changes along a sprouting vessel. In the base and stalk, where endothelial cells are relatively quiescent, ninein was predominantly found at the centrosome, whereas in the actively migrating tip cell the localization was strongly cytoplasmic. This is consistent with a model in which the MT-anchoring activity is decentralized in moving cells to allow for forward movement of the leading edge, and strongly suggests that ninein has an essential role in vessel tubulogenesis via effects on migrating tip cells (Figure).

The high levels of cytoplasmic ninein in tumor vessels suggest that they are abnormal in maintaining an activated or migratory state after vessels have formed, and this may be exploited to target the tumor vessels. It may also be possible to move ninein back to the centrosome in tumor vessels and this may “normalize” them, although there is still much to understand about the regulation and function of ninein before this experiment could be attempted. Some intriguing questions remain. For example, it is not clear how cytoplasmic ninein facilitates forward migration of the endothelial tip cell, and exactly how it is trafficked in the endothelial cell. There clearly seems to be movement along MTs, but there are suggestions that some of the endothelial ninein is associated with other structures or unanchored. It is also not clear whether centrosomal ninein is important in nonmigrating endothelial cells, although by analogy with other cell types it should stabilize MT anchoring to form the microtubule-organizing center (MTOC) and stabilize spindles when cells divide. In this light, because MTOCs orient to a flow gradient, it will be interesting to see whether this process is ninein-dependent. It is also interesting that, from this study, endothelial cells seem more prone to have large reservoirs of cytoplasmic ninein than other cell types, and how this might affect the function of ninein in blood vessel formation and function is not clear. It will also be interesting to see how

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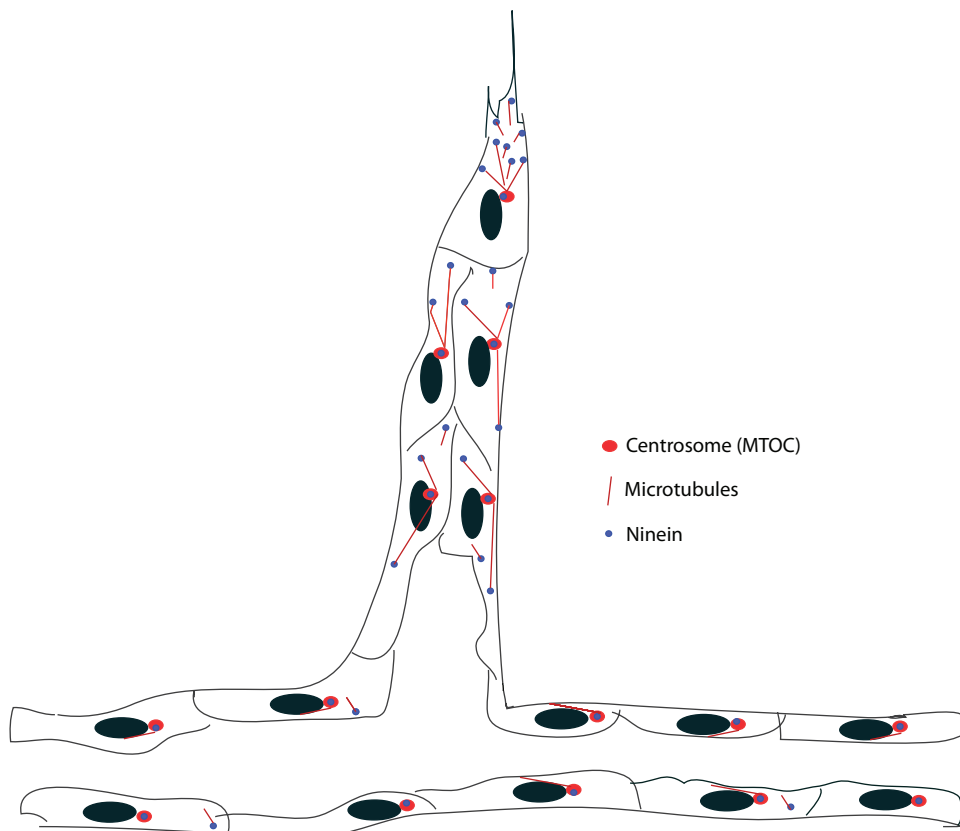
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**Figure.** Model of ninein function in endothelial cells. Ninein (blue circles) nucleates and anchors MTs (red lines) both at the centrosome (red circles) and away from the centrosome. The noncentrosomal location of ninein is enhanced in endothelial sprouts, especially in the tip cell.

ninein dynamics link to growth factor signaling, and the in situ localization of ninein tyrosine phosphorylation to cytoplasmic locations noted in this study is a good starting point.

This is the first report of a role for ninein in blood vessel formation, and the fact that it regulates MT dynamics and function makes a strong link between growth factor signaling and the cell behaviors that are elicited when this signaling is activated. This is an area of active interest in the field, because we have identified many of the “players” important in vessel formation and function, but we do not yet know the “game plan” of how these signals integrate and coordinate cellular behaviors to produce the appropriate outcomes. A protein such as ninein, which intimately associates with and regulates the microtubule cytoskeleton and dynamics, is a likely target for VEGF effects on endothelial cells.

## Disclosures

None.

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