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Developmental Cell
Preview

N-WASP Guides Cancer Cells toward LPA

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The actin remodeling factor N-WASP is best known as an Arp2/3 complex activator in processes like endocytosis, extracellular matrix degradation, and host-pathogen interaction. In this issue of *Development Cell*, Juin et al. establish a novel trafficking function for N-WASP in driving lysophosphatidic acid-dependent chemotaxis and metastasis of pancreatic cancer cells.

Dynamic movements of cell surfaces, their organelles, and whole cells within tissues depend on remodeling of actin filament bundles and networks capable of developing pushing and pulling forces. In most cases, pushing occurs through the addition of actin monomers—termed polymerization—onto filaments organized in bundles or networks, and pulling is mediated by gliding of myosin molecules along filament sides. These processes are regulated by intricate signaling pathways and multiple actin binding proteins gathering together into large molecular assemblies that control the assembly and turnover of arrays of actin filaments.

A key step in actin remodeling is nucleation of actin filaments. The Arp2/3 complex and its activators generate rapidly growing actin filaments through filament branching (Molinie and Gautreau, 2018). In mammals, activation of Arp2/3 complex-dependent actin filament branching is driven by eight class I nucleation promoting factors (NPFs). An important NPF is the widely expressed N-WASP, the closest relative of the largely hematopoietic WASP (Wiskott-Aldrich-Syndrome protein). N-WASP and WASP have similar domain organization and share important regulatory features and interaction partners, such as the essential Rho-family GTPase Cdc42, phospholipids like PIP_2 (phosphatidylinositol-4,5-bisphosphate), and adaptor proteins such as Nck or Grb2. N-WASP is autoinhibited through an intramolecular interaction, and synergistic activation by aforementioned interactors relieves this inhibition. N-WASP is essential for embryonic development in the mouse and plays a critical role in actin assembly processes at plasma membranes accompanying receptor-mediated endocytosis or in the protrusion of invadopodia (Molinie and Gautreau, 2018), specialized adhesion structures in cancer cells driving the degradation of and thus invasion into extracellular matrices. Notably, N-WASP and/or WASP is also hijacked by bacterial or viral pathogens to promote their adhesion to host cells or their dissemination. Nevertheless, in spite of decades of impressive research on the biochemistry and molecular regulation of N-WASP, a full understanding of the precise cellular functions of this protein, in particular in the context of distinct cell types and tissues, is still lacking (Li and Brakebusch, 2019).

The Machesky lab and colleagues now add a completely new twist to the story by establishing how N-WASP can contribute to the chemotactic migration and metastasis of cancer cells. They use beautiful murine genetics to induce pancreatic ductal adenocarcinoma (PDAC) in the absence or presence of N-WASP and show that the absence of N-WASP is sufficient to significantly extend the survival of these mice and to decrease tumor metastasis into neighboring tissues. Furthermore, isolation of these tumor cells and their characterization in tissue culture allowed the authors to shed light on the reasons underlying reduced tumor metastasis *in vivo*. Cells lacking N-WASP showed significantly impaired chemotactic migration toward LPA, gradients of which were previously shown to create a strong stimulus for melanoma cells to leave their primary tumor (Muinonen-Martin et al., 2014). N-WASP-dependent chemotaxis of PDAC cells was abrogated by inhibition of LPAR1 (LPA receptor 1). More surprisingly, the authors found that N-WASP loss of function interfered with LPAR1 recycling through Rab11-associated recycling endosomes, which promote efficient, LPA-mediated signaling and chemotaxis (Figure 1). N-WASP was also found to form a novel complex in this process with sortin nexin 18 (Snx18). Since LPA is prominently connected to activation of RhoA, followed by induction of myosin II-dependent contractility, the authors explored how RhoA signaling was affected by N-WASP. The presence of N-WASP coincided with increased RhoA activation by LPA-containing serum compared to cells lacking N-WASP. Lack of N-WASP also caused changes to cells' mechanical properties, as N-WASP-deficient cells exerted less force onto micropillars and displayed less force-dependent remodeling of extracellular matrices such as native collagen fibers. Together, these results imply that, independent from its role in endocytosis or protrusion formation, N-WASP significantly contributes to the efficiency of chemotactic migration by promoting LPA-dependent signaling to RhoA activation and contractility.

The novelty and originality of the results described by the authors provoke a number of immediate, mechanistic questions: How is N-WASP regulated in this process? Is it allosterically activated? And if so, does this lead to Arp2/3 complex-dependent actin assembly? Notably, increases in cellular PIP_2 levels are known to promote N-WASP-dependent vesicle motility (Benesch et al., 2002), and Cdc42 function was more recently linked to Rab11-charged,

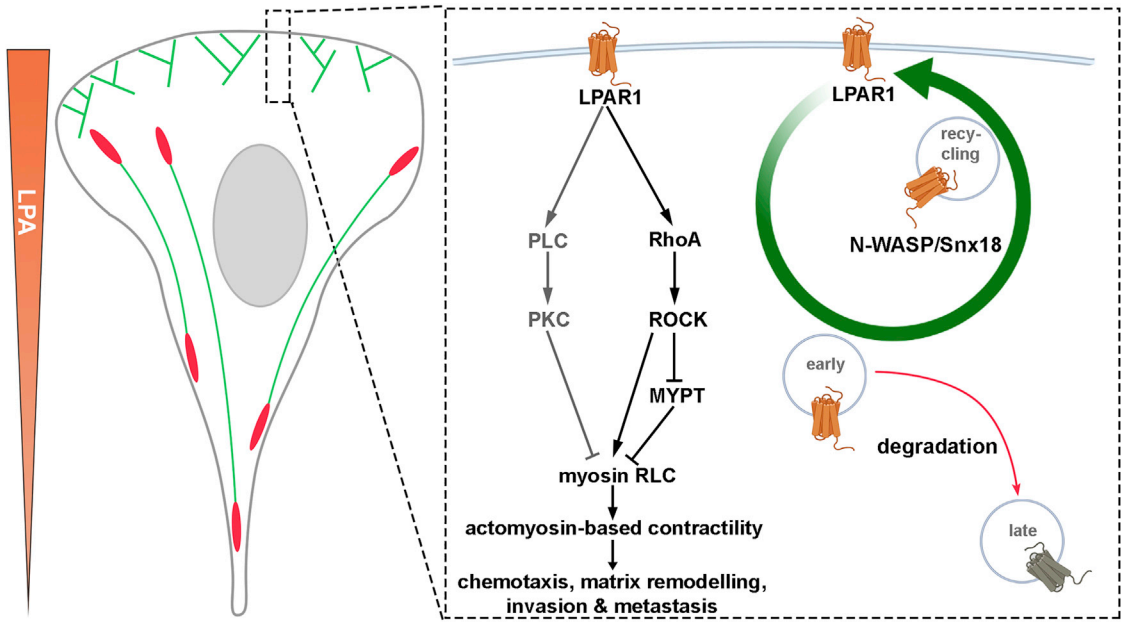

Figure 1. N-WASP in LPA-Induced Signaling and Cancer Cell Migration
Summary of results presented by Juin et al. (2019) and their implications for chemotactic migration and metastasis toward LPA gradients (cell on the left). LPAR1 signaling stimulates RhoA-dependent contractility by canonical, activatory signaling, either directly through ROCK (Rho-kinase) or indirectly through inhibition of MYPT (myosin light-chain phosphatase), a negative regulator of myosin RLC. LPA signaling to contractility is amplified by a novel, N-WASP/Snx18-dependent recycling loop of LPAR1 (green arrowed circle), preventing LPAR1 from premature degradation (red). Endosome stages are designated as early, recycling, and late endosomes (light gray). Generation of an inverse myosin II activity gradient through PLC and PKC signaling, as reported by others for both PDGF and LPA-dependent mesenchymal cell chemotaxis (Asokan et al., 2014, 2018), might add to the efficiency of myosin II-based cancer cell migration and metastasis toward LPA (dark gray).

recycling endosomes, at least in synapses (Rodal et al., 2008). The Bear lab has now firmly established that the Arp2/3 complex is largely dispensable for mesenchymal cell chemotaxis, toward both receptor tyrosine kinases like PDGF (Asokan et al., 2014) and more recently even LPA (Asokan et al., 2018). If this is also true for the PDAC cells used here, it would argue against N-WASP activating the Arp2/3 complex in driving LPA-mediated chemotaxis. Interestingly, *wasp* gene duplication in *Drosophila* has given rise to an actin polymerase called WHAMY, which lacks domains for Arp2/3 complex activation but associates with actin-enriched, Rab11-coated vesicles. While there is no known WHAMY ortholog in mammals and in light of the results in Juin et al. (2019), it is tempting to speculate that WHAMY's functions in flies might be played by WASP proteins on our side of the evolutionary tree. Future studies should also clarify how the N-WASP functions observed in Juin et al. (2019) might relate to those of the various additional, mammalian Arp2/3 activators, many of which are clearly involved in different types of membrane trafficking processes (Molinie and Gautreau, 2018). For instance, WASH protein levels were previously observed to be doubled upon N-WASP removal (Hänisch et al., 2010), potentially impacting the authors' observations if this is conserved in their system.

A final word on myosin and contractility in directed cell migration: as opposed to just a global increase in myosin II activity, James Bear and colleagues have recently described that PDGF-dependent chemotaxis requires the establishment of a negative gradient of myosin II activity throughout cells. This was clearly shown to be mediated by PLC (Phospholipase C) signaling and consequent inhibitory phosphorylation of myosin RLC (regulatory light chain) by PKC (protein kinase C) (Asokan et al., 2014). Although PLC and PKC isoforms are well-established LPA signaling mediators (Sheng et al., 2015), the impact of inhibitory phosphorylation of the myosin RLC and its impact on LPA-dependent, mesenchymal cell chemotaxis have only now been described in a recent preprint (Asokan et al., 2018). Considering these inhibitory phosphorylations might help interpret the puzzling, constitutive increase of activating phosphorylation of myosin RLC upon N-WASP removal observed in Juin et al. (2019). Clarifying all these issues requires further mechanistic study, but the exciting data described by Juin and colleagues uncover important novel aspects of N-WASP function in the context of cell contractility and trafficking, paving the way toward a more complete understanding of physiologically and disease-relevant migration processes such as cancer metastasis.

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