In This Issue

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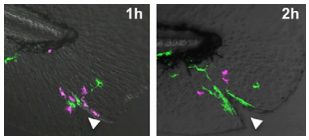
Macrophages show neutrophils the exit



Time-lapse images show macrophages (green) contacting neutrophils (magenta) and chasing them away from a wound (arrowhead).

Tauzin et al. describe how macrophages resolve inflammation by inducing neutrophils to leave wounded tissue.

Zebrafish neutrophils are attracted to wounds by reactive oxygen species (ROS) that activate the Src family kinase Lyn. Neutrophil-mediated inflammation is partly resolved by apoptosis and the cells' subsequent engulfment by macrophages. But neutrophils can also elect to leave wounded tissue in a process known as reverse migration. Whether macrophages promote this mode of inflammation resolution is unknown.

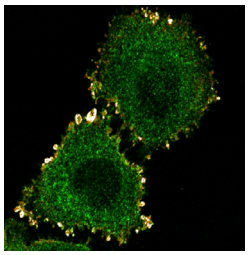
Tauzin et al. found that neutrophils were generally recruited to wounds before macrophages, but, once they arrived, macrophages often contacted neutrophils and appeared to shepherd them away from the damaged tissue. Neutrophils remained in wounds for longer times in zebrafish larvae lacking macrophages, the researchers discovered.

Like neutrophils, macrophages were attracted to wounds by ROS and Src family kinase signaling. Macrophages lacking the p22phox subunit of NADPH oxidase complex 2 (Nox2) or the tyrosine kinase Yrk were unable to migrate into wounds and induce the departure of neutrophils.

Mutations in the human homologue of *NOX2* cause chronic granulomatous disease, one symptom of which is enhanced neutrophil-mediated inflammation. Tauzin et al.'s findings suggest that this may be due to defects in macrophage recruitment and the induction of neutrophil reverse migration. Senior author Anna Huttenlocher now wants to investigate how macrophages drive neutrophils out of wounded tissue. She thinks the process may involve a combination of contact repulsion and chemokine signaling.

Tauzin, S., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201408090.

CDK5 opens the way for DLC1



DLC1 (red) and CDK5 (green) colocalize (white) at the focal adhesions of non-small cell lung cancer cells.

Tripathi et al. describe how the kinase CDK5 promotes the activity and correct localization of the tumor suppressor DLC1.

DLC1 is down-regulated in a wide variety of tumors. The protein localizes to focal adhesions and contains a C-terminal GAP domain that inactivates Rho GTPases. How DLC1's localization and activity are regulated is unknown, however.

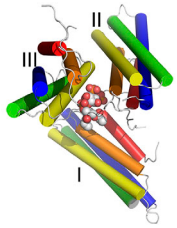
Tripathi et al. discovered that CDK5 phosphorylates four serine residues in the N-terminal half of DLC1. Mutating these serines to nonphosphorylatable alanine residues inhibited DLC1's ability to inactivate Rho and prevented the tumor suppressor's localization to focal adhesions by inhibiting its interactions with the adhesion proteins talin and tensin.

CDK5 phosphorylation disrupted an interaction between DLC1's N-terminal and GAP domains, suggesting that the kinase activates DLC1 by inducing its transition from a closed to an open conformation. Blocking this activation step reduced DLC1's tumor suppressor functions; unlike the wild-type protein, nonphosphorylatable DLC1 was unable to suppress cell migration or inhibit the growth of tumors in vivo.

Although CDK5 suppresses tumor migration and growth by activating DLC1, the kinase itself can also promote the growth of some human tumors, presumably by phosphorylating other target proteins. Tripathi et al. found that poorly differentiated lung tumors often showed high CDK5 and low DLC1 expression, which might allow CDK5's pro-oncogenic activities to predominate. Indeed, lung cancer cells lacking DLC1 grew in a CDK5-dependent manner, whereas cells expressing DLC1 grew independently of the kinase. Senior author Doug Lowy now plans to investigate other kinases that appear to oppose CDK5 and inactivate DLC1.

Tripathi, B.K., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201405105.

PIP_2 directs vinculin assembly



PIP_2 (red, orange, and white spheres) sits between the tail domains of three vinculin molecules (labeled I–III).

Chinthalapudi et al. reveal how the phospholipid PIP_2 induces oligomerization of the focal adhesion protein vinculin to promote adhesion turnover and cell migration.

Vinculin stabilizes nascent focal adhesions between the cell and ECM by linking them to the actin cytoskeleton. Vinculin also binds to phosphatidylinositol 4,5-bisphosphate (PIP_2), which is enriched at focal adhesions, but how this phospholipid regulates vinculin's function is unclear.

Chinthalapudi et al. obtained a crystal structure of vinculin's C-terminal tail bound to PIP_2 and found that the phospholipid was sandwiched between three vinculin molecules, inducing a subtle conformational change that promoted vinculin oligomerization. A previous study suggested that PIP_2 prevents vinculin from binding to F-actin, but Chinthalapudi et al.'s structure revealed that vinculin's PIP_2- and actin-binding sites are distinct, suggesting that vinculin may be able to bind to both molecules simultaneously.

The researchers identified single point mutations that inhibited vinculin's ability to bind PIP_2 without affecting the protein's interaction with actin. Although these PIP_2 binding-deficient mutants localized to focal adhesions, they were unable to rescue the disorganized actin filaments formed in vinculin-deficient fibroblasts. Moreover, whereas vinculin-null cells migrate faster than wild-type cells, fibroblasts expressing PIP_2-binding mutants moved very slowly, suggesting that their adhesions might be hyperstabilized. Indeed, photobleaching experiments demonstrated that vinculin mutants unable to bind PIP_2 were immobilized at focal adhesions, indicating that PIP_2 is required for vinculin turnover. Senior author Tina Izard now wants to investigate whether PIP_2 also promotes the oligomerization and function of other actin-binding proteins.

Chinthalapudi, K., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201404128.