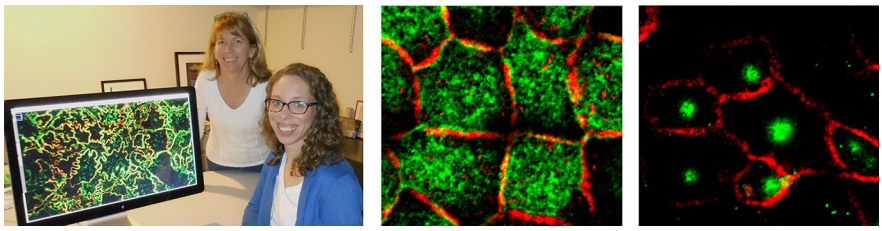
Text by Ben Short
bshort@rockefeller.edu

Merlin casts its spell on the cortical cytoskeleton

The tumor suppressor Merlin/NF2 inhibits the proliferation of confluent cells by regulating tension at the apical cell cortex.

FOCAL POINT



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Andrea McClatchey (left), Christine Chiasson-MacKenzie (right), and colleagues describe a novel mechanism by which the tumor suppressor Merlin/NF2 mediates contact-dependent inhibition of proliferation. By limiting the recruitment of the actin-binding protein Ezrin to the apical cell cortex, Merlin regulates the mechanical forces transduced between cell–cell contacts and the cortical cytoskeleton, allowing the EGFR to be immobilized and inhibited when epithelial cells reach confluency. In wild-type cells (center), myosin IIA (green) is uniformly distributed across the apical cortex, but in the absence of Merlin (right), it coalesces into puncta, an indicator of increased contractility and excess tension on cell–cell junctions (red).

Cells usually stop dividing once they are tightly packed in a tissue with plenty of neighboring cells contacting them. Tumor cells, however, overcome this contact-dependent inhibition of proliferation and continue to divide in response to growth factors present in their microenvironment. The tumor suppressor Merlin, also known as neurofibromatosis type 2 (NF2), prevents epithelial cells from responding to epidermal growth factor (EGF) once they are part of a confluent monolayer, but exactly how Merlin does this remains unclear. Chiasson-MacKenzie et al. now reveal that Merlin regulates the mechanical forces generated at the cortex of confluent epithelial cells, trapping the EGF receptor (EGFR) at the cell surface so that it can no longer stimulate proliferation (1).

In 2007, Andrea McClatchey and colleagues at Massachusetts General Hospital and Harvard Medical School discovered that, in confluent epithelial cells, Merlin inhibits EGFR internalization, thereby blocking the receptor's ability to stimulate cell division (2). Merlin is closely related to the Ezrin, Radixin, and Moesin (ERM) family of proteins that link the cortical actin cytoskeleton to the plasma membrane and, even though Merlin itself likely associates with actin indirectly, its ability to inhibit EGFR endocytosis depends on its localization to the cell cortex (3). Because the cortical cytoskeleton can regulate the lateral mobility of cell surface proteins, McClatchey and colleagues, led by postdoc Christine Chiasson-MacKenzie, decided to investigate whether Merlin inhibits EGFR by regulating the receptor's mobility in the membrane of confluent cells (1).

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Using single-particle tracking microscopy to follow the movements of individual receptor molecules, Chiasson-MacKenzie et al. found that EGFR became immobilized at the surface of wild-type liver epithelial cells once they became confluent, and that this immobilization depended on the cortical cytoskeleton. In Merlin-deficient cells, however, EGFR was mobile in both confluent and nonconfluent cells, and a Merlin mutant unable to localize to the cell cortex was unable to restore the receptor's immobilization. "That really taught us that Merlin controls the EGFR from the cortical cytoskeleton," McClatchey says.

Merlin also localizes to intercellular adherens junctions, which are mechanically coupled to the cortical cytoskeleton. Because Merlin is required for adherens junction integrity in many cell types (4), Chiasson-MacKenzie et al. expected them to be weaker, and under reduced tension, in Merlin-deficient liver cells. "But we got the opposite result," Chiasson-MacKenzie recalls. "The junctions were actually under increased mechanical tension in the absence of Merlin."

This suggested that the cortical cytoskeleton was reorganized in the absence of Merlin, causing it to pull harder on intercellular adhesions. Indeed, whereas myosin IIA was distributed uniformly across the apical cortex of wild-type epithelial cells, it coalesced into discrete puncta in Merlin-deficient cells, a telltale sign of increased apical contractility.

What could be causing this cortical reorganization? McClatchey and colleagues previously found that Merlin limits the recruitment of Ezrin to the cell cortex (5) and, sure enough, excess Ezrin accumulated at the apical cortex of cells lacking Merlin. Knocking down Ezrin restored myosin IIA's uniform distribution and reduced tension levels in Merlin-deficient cells.

The team's results suggested that, when cells grow to confluence, mechanical forces must be correctly balanced across the apical cortex and cell–cell junctions in order to immobilize the EGFR and limit its internalization and signaling capacity. Accordingly, treating confluent wild-type epithelia with the myosin II inhibitor blebbistatin prevented EGFR immobilization and permitted the receptor's continuing internalization. Mechanical forces—regulated by Merlin and cell–cell contact—therefore modulate EGFR, and perhaps other growth factor receptor, signaling. This could have important implications for development and disease. Tumors, for example, are often stiffer than their surrounding tissue, which could influence EGFR activity and contact-dependent inhibition of proliferation.

It remains to be seen how the cortical cytoskeleton immobilizes and then blocks the internalization of the EGFR. Intriguingly, immobilization is also regulated by the receptor itself. "We think this is a dynamic process that allows for the precise control of receptor signaling," Chiasson-MacKenzie says.

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3. Cole, B.K., et al. 2008. *Mol. Cell. Biol.* 28:1274–1284.
4. Gladden, A.B., et al. 2010. *Dev. Cell.* 19:727–739.
5. Hebert, A.M., et al. 2012. *Genes Dev.* 26:2709–2723.