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Cdc42 helps cancer cells make their exit

The Rho GTPase promotes metastasis by regulating the expression of β1 integrin.

To initiate a metastatic tumor in a new tissue, a cancer cell circulating in the blood must attach to the endothelial cells lining the blood vessel, squeeze between them, and then penetrate the underlying basement membrane. Reymond et al. reveal that the Rho GTPase Cdc42 drives this process—known as transendothelial migration—by promoting the expression of β1 integrin (1).

Rho GTPases regulate cell migration and invasion by controlling the dynamics of cell adhesions and the actin cytoskeleton (2). Their expression is often altered in tumors, and several Rho family members have been implicated in metastasis, though it's unclear which steps of the process they regulate (3). "We wanted to look at how Rho GTPases affected cancer cells' interactions with the endothelium," explains Anne Ridley, from King's College London, "because we think that this could be a rate-limiting step of metastasis that could be targeted in cancer."

In an siRNA screen of human Rho GTPases, Ridley and colleagues, led by postdoc Nicolas Reymond, found that the depletion of several Rho family members inhibited prostate cancer cells' attachments to an endothelial monolayer (1). "We made movies of the knockdown cells and noticed that, although quite a lot of the Rho GTPases affected adhesion, it was only Cdc42 depletion that dramatically impaired the subsequent intercalation between endothelial cells," Ridley recalls. "Cancer cells lacking Cdc42 just sat on top of the monolayer and didn't invade into the endothelium."

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Invading cancer cells send out protrusions that reach down between neighboring endothelial cells to contact the underlying basement membrane. The cancer cells then spread out on this extracellular matrix (ECM) so that the endothelial cells retract and allow the invaders to intercalate between them (4). In the absence of Cdc42, however, cancer cells failed to spread out on the basement membrane, and Cdc42-deficient cells showed reduced adhesion to ECM-coated coverslips.

Cdc42 therefore promotes the attachment of cancer cells to both endothelial cells and the underlying basement membrane during transendothelial migration. But how does Cdc42 regulate cancer cell adhesion? Reymond et al. found that the GTPase drives expression of β1 integrin, an adhesion receptor known to be involved in metastasis (5). "The levels of β1 integrin were reduced [in Cdc42-deficient cells]," Ridley explains. "β1 integrin is important for adhesion to the ECM and could be important for the initial attachment to endothelial cells as well."

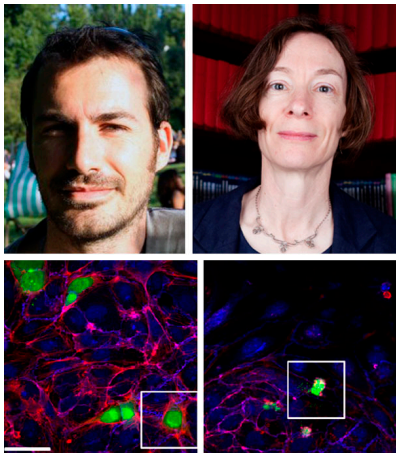
Knocking down β1 integrin inhibited cancer cell intercalation and transendothelial migration, whereas overexpressing the integrin in Cdc42-deficient cells restored endothelial invasion. Cdc42 promoted β1 integrin expression by activating a transcription factor called SRF. A constitutively active form of the transcription factor was also capable of restoring endothelial intercalation to cancer cells lacking Cdc42.

Reymond et al. then investigated how Cdc42 affects cancer cells' behavior in vivo, injecting both control and Cdc42-deficient cancer cells into mouse tail veins. Within minutes, both cell types accumulated in the lung vasculature, but the control cells spread out more on the vessel endothelium. Over the next few hours, control cells persisted in the lung, whereas the numbers of Cdc42-deficient cells declined. "And six weeks later, the control cells had generated more metastases compared to the Cdc42-knockdown cells," Ridley says.

Importantly, Cdc42 was only knocked down transiently in these experiments—levels of the GTPase were back to normal after three days—suggesting that blocking cancer cells' initial interactions with endothelia is sufficient to inhibit the subsequent growth of tumor metastases. This could represent a new therapeutic approach, though Ridley says that Cdc42 itself is unlikely to be a viable drug target. "One possibility is to target β1 integrin. Or you could target something downstream of Cdc42 that links it to SRF. Our next goal is to find out which Cdc42 effectors are involved in this pathway."

1. Reymond, N., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201205169.
2. Vega, F.M., and A.J. Ridley. 2008. *FEBS Lett.* 582:2093–2101.
3. Hall, A. 2009. *Cancer Metastasis Rev.* 28:5–14.
4. Reymond, N., et al. 2012. *Methods Mol. Biol.* 827:123–142.
5. Wang, H., et al. 2004. *J. Cell Biol.* 164:935–941.

Focal Point



PHOTOS COURTESY OF NICOLAS REYMOND, UK ACADEMY OF MEDICAL SCIENCES

Nicolas Reymond (top left), Anne Ridley (top right), and colleagues (not pictured) reveal that the small GTPase Cdc42 promotes the migration of metastasizing cancer cells out of blood vessels by inducing the expression of β1 integrin. In the absence of Cdc42 or β1 integrin, cancer cells show reduced adhesion to both endothelial cells and their underlying basement membrane, thereby blocking transendothelial migration. In vitro, control cancer cells (green, bottom left) intercalate between endothelial cells stained for actin (red) and intercellular junctions (blue), whereas Cdc42-deficient cancer cells (bottom right) remain on top of the monolayer. In mice, transient depletion of Cdc42 inhibits cancer cells' ability to colonize and form metastases in the lung.