Ubiquitin-Mediated Suppression of Cellular Transformation

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While protein construction is crucial for all cells, properly dismantling proteins is just as important. To degrade proteins, cells use a protein apparatus called the proteasome. Proteins are often targeted to the proteasome via the attachment of ubiquitin molecules. Ubiquitin molecules are attached to proteins via the successive action of three families of proteins: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3). Ubiquitin-mediated protein turnover is important in cell cycle control, and mutations in ubiquitin pathway components have been identified in many cancers (Mani and Gelman, 2005). Studying how ubiquitin pathway proteins regulate cell growth thus has important implications for understanding tumorigenesis.

Cullin RING ligases (CRLs) make up the largest class of E3 ubiquitin ligases. CRL targets include both tumor suppressors and oncoproteins, thus they can both inhibit and promote oncogenic transformation. Consistent with this, the genes that encode CRLs are often mutated in cancer (Lee and Zhou, 2010). To better understand how CRLs are involved in cancer, postdoctoral fellow Dr. Anjali Teckchandani and colleagues in the laboratory of Dr. Jonathan Cooper (Basic Sciences Division) dissected the mechanism by which the CRL Cullin5 (Cul5) influences cellular transformation. They found that Cul5 destabilizes p130 Crk-associated substrate (p130Cas), a target of phosphorylation by the Src tyrosine kinase, a well-known oncoprotein, leading to inhibition of Src-driven cellular transformation. "Given that the Src kinase is activated in many human mammary epithelial tumors, we may have uncovered a new tumor suppressor mechanism," says Dr. Cooper.

The researchers first reduced the expression of Cul5 via a short-hairpin RNA (shRNA) in the MCF10A breast cell line. To test for cell transformation, they assayed colony formation. Cul5-deficient cells generated dysmorphic colonies and displayed increased proliferation and reduced apoptosis relative to a Cul2 knockdown control. It was also found that Cul5 depletion increased cell motility. Cells with reduced levels of Cul5 also showed morphological abnormalities, including elongated lamellipodia and membrane ruffling.

In previous work, the researchers showed that Cul5 is necessary for the degradation of activated Src kinase (Laszlo and Cooper, 2009), a known oncoprotein. Therefore, they tested whether Src was required for transformation of Cul5-deficient cells. Src protein levels were increased in Cul5-deficient cells, and Src activity, as measured by autophosphorylation at tyrosine 416, was also increased. Both shRNA knockdown and pharmacological inhibition of Src suppressed the transformation of Cul5-deficient cells. The researchers hypothesized that other substrates of Cul5 might be phosphorylated by Src and thus contain phosphotyrosine (pY). The knockdown of several SOCS adapter proteins, which recognize pY, recapitulated the effects of Cul5 knockdown on cell transformation, suggesting cooperation between Cul5 and SOCS adaptor proteins. The researchers then used mass spectrometry to identify pY peptides from control, Cul2-, and Cul5-deficient cells. Eighteen pY peptides corresponding to p130Cas were detected in the Cul5 knockdown sample, but not controls. The researchers confirmed that p130Cas levels were increased in Cul5-deficient cells and in cells with SOCS protein knockdown. Knockdown of p130Cas revealed that this protein was required for transformation of Cul5-deficient cells. Co-immunoprecipitation experiments confirmed a physical interaction between Cul5 and p130Cas and also showed that SOCS6 was the only SOCS adapter protein bound to p130Cas in MCF10A cells, suggesting the existence of a ternary complex between Cul5, SOCS6, and p130Cas.

"Like Src, Cas is also activated in human mammary carcinomas and contributes to invasion and resistance to anti-estrogens. So, the Cul5 ubiquitin ligase inhibits transformation by targeting Cas for ubiquitylation and degradation," says Dr. Cooper. Future studies are aimed at the role of Cas ubiquitylation in the regulation of cell migration, which may lead to insights into how cancer cells metastasize.

Teckchandani A, Laszlo GS, Simó S, Shah K, Pilling C, Strait AA, Cooper JA. 2013. Cullin5 destabilizes Cas to inhibit Src-dependent cell transformation. J Cell Sci Epub 27 Nov 2013.

See also: Mani A, Gelmann EP. 2005. The Ubiquitin-Proteasome Pathway and Its Role in Cancer. J Clin Oncol 23(21):4776-4789

Lee J, Zhou P. 2010. Cullins and Cancer. Genes Cancer 1(7):690-699.

Laszlo GS, Cooper JA. 2009. Restriction of Src activity by Cullin-5. Curr Biol 19(2):157-162.

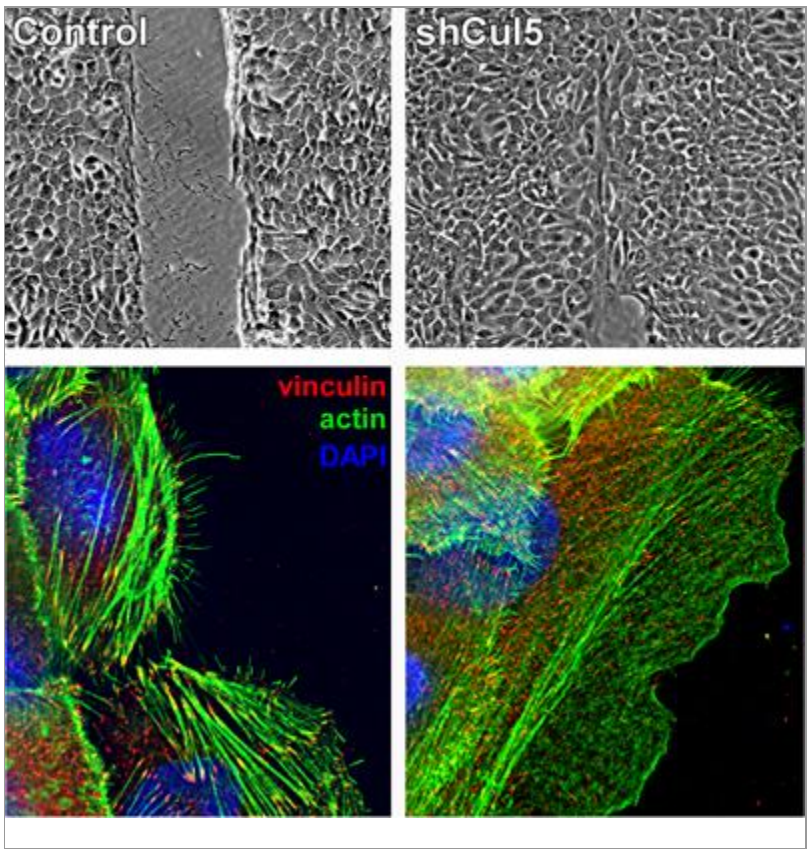

Image provided by Dr. Anjali Teckchandani.

shRNA-mediated knockdown of Cullin5 (shCul5) results in increased migration of cells into a scratch wound (top right), an elongated leading lamellipodium (bottom right, green), and small focal adhesions (bottom right, red).