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Actin-Based Protrusions: Promoters or Inhibitors of Cancer Invasion?

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In a recent issue of *Cell*, Silva and colleagues reported the identification of CYFIP1, a member of the actin-assembly-promoting Scar/WAVE complex, as an invasion suppressor in epithelial cancers. This study challenges ideas about the role of actin in cancer invasion.

Cancer progression is modulated by a balance of oncogenes and tumor suppressors. Oncogenes promote tumor formation and growth by a whole host of mechanisms, including allowing increased proliferation, reducing cell death, and allowing adhesion-independent cell survival. Tumor suppressors, on the other hand, prevent tumor growth and progression by opposing these phenomena and also by promoting the normal differentiated state of a tissue. Cancer progresses partly by deleting or suppressing the expression or activity of tumor suppressors. Tumors generally have unique and evolving gene expression signatures, and it is likely that the human genome contains several undiscovered tumor suppressors. Identification of new tumor suppressors and oncogenes is a major goal of cancer research, as they may represent new drug targets.

It was with this aim in mind that Silva and colleagues used RNAi to target genes found in regions of chromosomes that are frequently deleted in tumors (Silva et al., 2009). They analyzed regions of deletion that contained no known tumor suppressors in hopes of finding new ones. Of 29 genes tested, CYFIP1 was required for normal epithelial morphology in organized mammary cell clusters (acini) formed in 3D matrix.

CYFIP stands for cytoplasmic FMR1 interacting protein, as the CYFIPs were discovered as proteins that interact with the fragile-X syndrome mental retardation (FMR) proteins. There are two known CYFIP proteins in humans, CYFIP1 (also p140Sra) and CYFIP2 (also PIR121). The CYFIP gene is also found at a breakpoint hotspot for the Prader-Willi/Angelman syndromes and may be implicated in autism (Sahoo et al., 2006). The role of CYFIP1 in these disorders is not well understood, but CYFIP1 binds directly to the translation initiation factor eIF4E and represses translation of FMRP targets in the brain (Napoli et al., 2008). Thus, altered protein translation likely contributes to these syndromes. However, CYFIP1 also regulates actin cytoskeletal architecture, as it is an integral member of the Scar/WAVE complex. It is not clear whether protein translation regulation by CYFIP1 is influenced by or completely independent of the actin cytoskeletal architecture. However, Silva and colleagues concluded that FMR1-CYFIP1 was not involved in tumor cell invasion, as knockdown of FMR1 did not affect morphology of spheroid cultures.

The Scar/WAVE complex is a conserved complex of 5 subunits that stimulates actin assembly when cells form lamellipodia (Figure 1A). The subunits appear to depend largely on the integrity of the whole complex for their stability, as knockdown or knockout of individual subunits often leads to degradation of the whole complex (Blagg and Insall, 2004). The Scar/WAVE complex exists in an inactive state and is activated by signals to the small GTPase Rac1 that trigger exposure of the Scar/WAVE C-terminal VCA domain (Figure 1A), leading to activation of the Arp2/3 complex and assembly of branched actin structures (Ismail et al., 2009). This actin assembly produces thin sheet-like extensions of the plasma membrane known as lamellipodia and may also cooperate with other actin regulatory proteins to assemble spiky protrusions known as filopodia.

Since cells use actin-mediated protrusions to migrate, it seems perplexing that a protein that promotes lamellipodia formation could act as a suppressor, rather than a promoter, of invasion. However, there are several possible explanations for these observations. Silva and colleagues demonstrated that actin assembly mediated by CYFIP1 is important for the formation of cell-cell and cell-matrix adhesions. The formation of cell-cell adherence junctions involves the interactions of cadherin proteins that span the plasma membrane and form homo-oligomers with cadherins on adjacent cells. For this process to occur normally, cells first contact each other and explore the contact surface using lamellipodia and filopodia assembled via Arp2/3 complex and Scar/WAVE-mediated actin assembly (Yamada and Nelson, 2007) (Figure 1B). Likewise, Arp2/3 complex and actin assembly are important for the formation of integrin-mediated contacts with the extracellular matrix (Serrels et al., 2007). Silva and colleagues demonstrate that loss of CYFIP1-mediated actin assembly disrupts the architecture of cells via destabilization of their interactions with each other and with the matrix. Thus, they conclude that lamellipodial and filopodial actin assembly are important for the establishment of normal epithelial architecture.

While disruption of normal tissue architecture is likely to promote a more invasive phenotype in transformed cells, it still might seem surprising that loss of lamellipodia and filopodia wouldn't lead to less invasive cells because of the expected reduction in motility. However, it is unclear what the real role of short-lived highly dynamic structures like lamellipodia and filopodia are in cancer cell invasion. Structures that are much longer lived, such as invadopodia, may be more relevant for cancer cell invasion, whereas filopodia and lamellipodia may be more

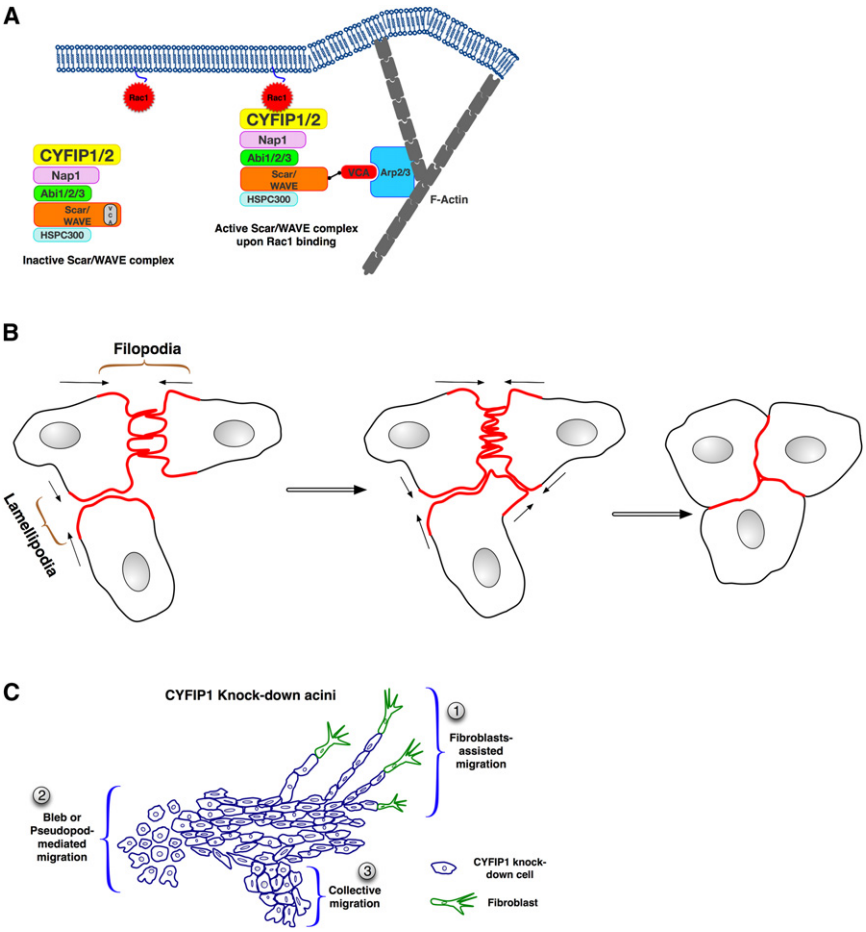

Figure 1. The Scar/WAVE Complex and Cell Invasion Mechanisms

(A) The inactive Scar/WAVE complex is activated upon Rac1 binding, which exposes the C-terminal VCA domain of Scar/WAVE leading to the Arp2/3 complex activation and assembly of new actin filaments. CYFIP proteins are the key component of Rac1 binding and complex stabilization. As a result of actin polymerization, the membrane is extended to generate lamellipodia and/or filopodia.

(B) When cells form cell-cell junctions, cells contact each other with lamellipodia and filopodia. Scar/WAVE complex and the Arp2/3 complex polymerize actin to drive this process (red outline). Extending lamellipodia or filopodia expands the contact surface between cells. Meanwhile, cadherin proteins accumulate at the cell-cell contacts. Finally, cadherins seal up the contact to generate cell-cell adherence junctions by forming homo-oligomers and anchoring actin to the junctions.

(C) Possible mechanisms for enhanced motility of CYFIP1 knockdown epithelial cells. (1) CYFIP1 knockdown cells may invade without lamellipodia or filopodia using fibroblast-assisted motility. (2) In addition, the knockdown cells could also invade using membrane bleb- or pseudopod-mediated mechanisms. These nonactin assembly based mechanisms allow migration of individual cells. (3) The cells could also invade collectively with a few cells moving with pseudopodia at the front.

important during normal morphogenesis and the formation of cell-cell contacts. Loss of CYFIP1 could lead to a more loosely packed tumor.

Cells without CYFIP1 might still be able to migrate and invade using collective migration and an alternative mode of motility that doesn't need lamellipodia or filopodia (Figure 1C). This reflects both the ability of other cells in the microenvironment, such as fibroblasts, to assist the motility of the cancer cells (Pinner and Sahai, 2008) and the ability of the cancer cells to use alternate types of protrusions, such as membrane blebs or pseudopod extensions to migrate (Sanz-Moreno et al., 2008). Could loss of Cyfip1 be tipping the balance between lamellipodial and bleb-mediated motility in these epithelial cells? Silva and colleagues coinjected fibroblasts together with keratinocytes to show that loss of Cyfip1 promoted invasion. Thus, the fibroblasts might assist in collective invasion by carving a path in the extracellular matrix and forming a leading front for the invading cells (Figure 1C). It has recently been demonstrated that normal breast development proceeds by a mechanism resembling collective migration and doesn't require lamellipodia and filopodia, so it would make sense if epithelial cancer cells could use a similar mechanism for invasion (Ewald et al., 2008).

In their discussion, Silva and colleagues conclude that the invasion suppression effect of Cyfip1 may be generally due to a loss in the regulation of actin dynamics. This interesting hypothesis raises many questions. It might be of interest to take a closer look at how actin dynamics are perturbed by loss of Cyfip1. Presumably, cells still have Cyfip2 and may, thus, still have functional Scar/WAVE complex. Does the Scar/WAVE complex have a positive role as a component of cell-cell junctions, or is it perhaps sequestered there once cell contacts are formed to prevent further lamellipodia actin assembly? What turns off Scar/WAVE once cells form stable contacts? Other members of the Scar/WAVE complex, Abi and Nap1, have a role in cell-cell junctions independently of Scar/WAVE proteins (Ryu et al., 2009), but it is unclear if Cyfip1/2 are found at cell-cell junctions. As imaging techniques and models for human cancers become more accessible, it is increasingly possible to ask fundamental questions about actin dynamics and its role in vivo, which opens up an amazing new dimension for future cancer research.

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Pancreatic Cancer—Could It Be that Simple? A Different Context of Vulnerability

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In a recent issue of *Science*, Olive and colleagues document that inhibition of Hedgehog (Hh) signaling in a genetically engineered mouse model of pancreatic cancer can enhance the intratumor concentration of certain anticancer drugs. Could this finding provide us with a new method to attack pancreatic cancer?

Pancreatic cancer remains a major challenge for all of us. It is the fourth leading cause of death from cancer in the US, with an estimated 37,680 people diagnosed with the disease and 34,280 people dying from the disease each year (Jemal et al., 2008). Worldwide, more than 213,000 are diagnosed with pancreatic cancer each year (Koorstra et al., 2008). It has the worst 1 and 5 year survival of any cancer. In addition to a poor survival rate, patients with pancreatic cancer have a great deal of suffering, with a particularly high incidence of pain—mostly caused by a predilection for the tumor to invade the perineural space of nerves in the celiac plexus (Zhu et al., 1999). In addition, substantial weight loss and multiple gastrointestinal symptoms sap the energy of patients with the disease. If the above description of the disease is not bad enough, there has recently been worse news (Jones et al., 2008). In a comprehensive genetic analysis of 24 patients' pancreatic cancers, the authors noted an average of 63 genetic alterations in each tumor, the majority of which were point mutations. However, these alterations did define a set of 12 recurrent pathways as possible ways to attack the disease; the findings remind us just how challenging pancreatic cancer is to treat.

It is a mystery as to why so many currently available anticancer agents with demonstrated antitumor activity in in vitro and in vivo tumor models do not work in patients with pancreatic cancer. Is it just because of the inherent resistance or heterogeneity of pancreatic cancer? Other tumors, such as colon and lung, have inherent resistance and heterogeneity, yet anticancer agents frequently cause tumor shrinkage and improve survival for patients with those diseases. Why is this?

It has been recognized for some period of time that pancreatic cancers often demonstrate hypoperfusion (Park et al., 2009) (Figure 1). Microscopically, almost a sine qua non of pancreatic cancer is the dense fibroinflammatory reaction that invariably accompanies the disease (Mahadevan and Von Hoff, 2007). This appearance is also noted with other types of cancer, such as breast cancer. Could it be so simple that hypoperfusion explains why any therapeutic agent simply cannot get to the tumor cells because the circulation to pancreatic cancer is so poor? Pancreatic cancer is one of the tumor types to be consistently hypoxic, possibly because of hypoperfusion, and it is notoriously resistant to antiangiogenic agents (Van Cutsem et al., 2009). If hypoperfusion is the reason (or at least one of the reasons) for the resistance of pancreatic cancers to our therapies, Olive and colleagues (2009) have now given us a new window on how the stroma (the fibroinflammatory component of the tumor) may be altered, possibly improving our ability to deliver anticancer therapies to the tumor cells.

In a series of well-strategized and careful pieces of work, Tuveson and colleagues have generated genetically engineered mouse models that closely mimic the human disease condition (Hingorani et al., 2003, 2005; Hruban et al., 2006). Of particular interest is that KPC mice, which conditionally express endogenous mutant Kras and p53 alleles in pancreatic cells, have, as a very early histologic feature of tumorigenesis, the appearance of a characteristic stroma with infiltration of regulatory T cells, fibroblasts, and a fibroinflammatory component.

In an important follow-up study, Olive and colleagues (2009) now demonstrate that an Hh-signaling pathway antagonist could be used to deplete tumor-associated stromal tissues and improve the delivery of one of the few modestly active anti-pancreatic-cancer agents, gemcitabine, into the pancreatic cancer. They first show that tumors in KPC mice had