the natural history of human tumors. First of all, the Cyclin D1 and Cdk4 mutations used in these studies were introduced in the germline, not in advanced tumors (RNAi results must be interpreted more cautiously, since other loci may be affected). More importantly, the etiology of experimental tumors differs significantly from that of human cancers. For instance, experimental mouse tumors often arise from tissues in which most cells carry the tumor-inducing mutation (MMTV-driven c-*neu*/*erbB-2* expression in the papers discussed here). In contrast, human tumors, especially solid tumors, result from mutations in single or few cells that accumulate additional mutations through a process of clonal selection. Thus, tumor development in human patients is likely to be less dependent on any given mutated gene than experimental tumors. Yet, these considerations should not be an excuse to further delay testing in patients suffering from HER-2-positive breast tumors the effectiveness of selective Cdk4/6 inhibitors, as suggested by these new studies (Landis et al., 2006; Yu et al., 2006).

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Fly Src: The Yin and Yang of tumor invasion and tumor suppression

The non-receptor tyrosine kinase Src is inactivated by the C-terminal Src kinase Csk. In a recent paper in *Developmental Cell*, Vidal et al. show that loss of *Drosophila* Csk (dCsk) in a large field of cells results in cell proliferation and disorganization of tissue architecture. In contrast, local inactivation of dCsk in a small field of cells results in loss of cells that are adjacent to normal tissue. This loss occurs by basal migration and death by apoptosis. These findings may shed light on mechanisms that restrain tumor initiation.

During the progression of many epithelial cancers, including breast and colon carcinomas, the expression and activity of the tyrosine kinase Src becomes progressively elevated. Src activity appears to be particularly important in tumor cell invasion and metastasis (Frame, 2002). Thus, inhibition of Src inhibits tumor metastasis in a number of xenograft models, as well as in transgenic mice overexpressing polyoma middle T or Her2. In cell culture, activated Src induces the appearance of invasive adhesions known as podosomes or invadopodia, sites of local matrix degradation. The pathway by which Src induces cell invasion through reconstituted basement membrane matrices involves the activation of the focal adhesion kinase (FAK) and the stress-activated Jun kinase (JNK), and the induction and local secretion of matrix metalloproteinases and other matrix-degrading proteases (Hsia et al., 2003). Activated Src is capable of inducing autonomous cell proliferation and cell transformation: viral *src* is a potent transforming gene. Yet mutations that activate Src are found rarely if at all in human cancers, and those that have been reported—the report has been disputed—occur in advanced metastatic cancers (Irby et al., 1999). The reason that activating mutations in Src do not appear to initiate human cancers is unknown.

One potential explanation for the rarity of activating mutations in Src is the ability of normal cells to suppress the malignant behavior of mutant cells within their midst. In cell culture, normal fibroblasts can suppress the transformed phenotype of Src-transformed fibroblasts, a phenomenon that does not appear to depend on junctional communication (Alexander et al., 2004). Similar tumor-suppressive mechanisms may operate in vivo. For example, normal liver tissue can induce the differentiation of injected hepatocarcinoma cells, provided that the tumor cells are present in small groups or single cells (McCullough et al., 1998). Similarly, the formation of tumors by grafted papilloma cells can be suppressed by admixture with normal keratinocytes (Strickland et al., 1992). Inhibition of tumor cell growth by adjacent normal cells has been postulated to represent a potent tumor suppression mechanism.

New light on these issues may now

have been shed by a recent study of Cagan and coworkers (Vidal et al., 2006). They used that well-known tumor model, the fruit fly *Drosophila*, to examine the results of Src activation in groups of adjacent cells. Local activation was achieved by the regulated expression of an RNAi construct that inhibited expression of the *Drosophila* homolog of Csk, dCsk. dCsk inhibits the activity of the Src isoform dSrc64B, and previous work from this group had shown that the effects of loss of dCsk are mediated by Src activation (Read et al., 2004). When dCsk expression was blocked in a large field of cells in the fly eye or wing, the entire structure became enlarged and disorganized. In the retina, the loss of dCsk led to an increase in cell number, as a result of both increased proliferation and decreased apoptosis. Furthermore, loss of dCsk and the consequent activation of dSrc suppressed apoptosis induced by expression of the apoptosis inducers Reaper and Hid. Mammalian Src is involved in mitogenic signaling by integrins and growth factor receptors, and it is well known that Src, although proapoptotic under some circumstances, can suppress apoptosis by inducing Ras-MAP kinase, PI-3-kinase-Akt, and Stat3 signaling. Thus, the mitogenic and antiapoptotic effects of *Drosophila* Src (presumably dSrc64B) came as no surprise.

In addition to its effect on cell proliferation and cell death, Src activation resulted in disruption of tissue architecture. In the normal eye, interommatidial precursor cells differentiate into pigment cells and sensory bristles that form an organized hexagonal lattice surrounding the individual facets or ommatidia. On suppression of Csk expression, the patterning of these cells became disorganized. Time-lapse movies revealed that this patterning defect was due not to a failure of cells to undergo the required movements, but to a failure to maintain correct positioning, possibly as result of a failure in cell adhesion. Both mammalian and *Drosophila* Src are known to decrease E-cadherin-dependent adhesion. Consistent with the idea that the patterning defect was due to inhibition of E-cadherin-mediated adhesion, the level of Armadillo (β-catenin) was decreased in dCsk-deficient cells, and the patterning defects due to dCsk deficiency (but not the tissue overgrowth) could be suppressed by coexpression of *Drosophila* E-cadherin. Again, the effects of Src activation in *Drosophila* seemed very consistent with its effects in mammalian cell culture systems.

The surprise came when discrete patches of dCsk-deficient cells surrounded by normal cells were generated. This was achieved by expressing the dCsk RNAi construct in clonal patches or with a promoter with a restricted expression domain. Unlike patches of cells generated by inactivation of many other fly tumor suppressors, these patches of dCsk-deficient cells did not overgrow the adjacent normal tissue. Instead, cells with activated Src at the patch boundary were basally extruded from the epithelium and underwent apoptotic cell death. Whether any of these cells were actually migrating into or across the basement membrane was not established in this study, and it would presumably require electron microscopy to resolve this question. Nevertheless, it is intriguing to note that the basal migration of the cells could be inhibited by reducing the expression of the *Drosophila* matrix metalloproteinase MMP2 or by coexpression of the tissue inhibitor of metalloproteinases (TIMP). Thus, like the invasive migration of mammalian Src-transformed cells through reconstituted basement membrane matrix, the basal migration of dCsk-deficient cells appeared to be dependent on matrix metalloproteinase function. Strikingly, therefore, when dCsk-deficient cells are adjacent to normal cells, the same functions that in mammalian cells are responsible for Src-induced cell invasion induce the basal migration of the cells and their death by apoptosis.

To explore the signaling pathways by which migration and apoptosis of Csk-deficient cells is induced when the cells are adjacent to normal cells, Vidal et al. examined the effects of inhibiting mediators of apoptotic signaling, cell migration, and E-cadherin-dependent adhesion. The *Drosophila* JNK ortholog dJNK has been implicated in the apoptotic elimination of clonal patches of cells that are mutant for the tumor suppressor *scribble* (Brumby and Richardson, 2003). Inhibiting dJNK activation (by overexpression of the dJnk-specific phosphatase Puckered or by coexpression of a dominant-negative dJnk) blocked the migration of dCsk-deficient cells, while promoting dJnk activation (by reducing the dose of Puckered) promoted migration and cell death. Overexpression of the *Drosophila* Rho protein Rho1 promoted cell migration and death, whereas a reduction in the dose of Rho1 decreased the migration and apoptosis of dCsk-deficient cells. Rho1 has also been shown to be required for the basal migration of *Drosophila* imaginal disc cells lacking Moesin, which display a phenotype similar to those of *dCsk* cells (Speck et al., 2003). The involvement of Rho1 is of particular interest, because it has been shown that the assembly and function of Src-induced podosomes in mammalian cells is dependent on Rho function (Berdeaux et al., 2004). Furthermore, in colon cancer cells Src-induced deregulation of E-cadherin is dependent on the Rho-regulated kinase ROCK (Avizienyte et al., 2004). The migration and apoptosis of *dCsk* cells from discrete patches was suppressed by reducing the dose of dE-cadherin, seemingly pointing to a positive role for dE-cadherin in basal migration and cell death. Finally, flies contain a homolog of the mammalian Src substrate p120-catenin, a component of E-cadherin-containing adherens junctions. A reduction in the expression of this protein, dP120ctn, also reduced the migration and apoptosis of *dCsk* cells. Thus, it appears that dJnk, Rho1, dE-cadherin, and dP120ctn all act positively in the pathways that induce cell migration and apoptosis.

What mechanisms can account for the selective migration and death of dCsk-deficient cells that are adjacent to normal tissue? It seems there are significant differences in the signaling pathways that induce proliferation and tissue disruption in broad fields of *dCsk* cells and those that induce migration and apoptosis in discrete patches. For example, although the disturbances in patterning resulting from inactivation of dCsk in a large field were suppressed by *overexpression* of dE-cadherin, the migration and apoptosis of *dCsk* cells from smaller fields were in contrast suppressed by *reducing* the dose of dE-cadherin. Reducing the dose of Rho1 or dP120ctn inhibited the basal migration of dCsk-deficient cells in discrete patches but had no effect on the phenotype of *dCsk* cells in large fields. On the basis of these observations, Vidal et al. propose a model in which the homotypic interactions between E-cadherin molecules on adjacent cells produce different signaling outcomes when both adjacent cells are dCsk-deficient and when one cell is dCsk-deficient while its adjacent neighbor is normal.

But perhaps modified versions of this model should also be considered. For example, another view is that the basal extrusion observed in *dCsk* patches might be a secondary consequence of the proc-

ess of apoptosis. If, for example, the *dCsk* cells in these patches proliferate more slowly than their neighbors, they might be eliminated by competition. This is the process by which rapidly growing cells eliminate more slowly growing neighbors by JNK-dependent apoptosis (Gallant, 2005); in this process the apoptotic cells are also basally extruded. It would thus be interesting to determine whether basal migration still occurs when apoptosis is suppressed. An alternative possibility is that the apoptosis is a consequence of basal migration. Epithelial cells are dependent on cell-cell and cell-matrix signals for their survival. Cells that migrate basally from their normal position might therefore undergo apoptosis because they are deprived of antiapoptotic signals present in their normal niche. Thus, on this view the death of the migratory cells might be secondary to their movement out of a protective niche within the intact epithelium. The finding that loss of MMP2 blocks both basal migration and cell death is consistent with this latter view.

Irrespective of the precise model invoked to explain these observations, it seems clear that tissue context can determine the outcome of Src activation. Some of the same signaling pathways and molecules that act to promote mammalian tumor development and metastasis can, at least in the fly, function to promote cell death when activated in small groups of cells. Clearly the question now is: does discrete activation of Src have similar effects in mammalian epithelia, and does this account for the failure of mutationally activated Src to initiate tumor formation in man? Introduction of a conditionally expressed allele of activated Src into the mouse genome might provide the answer.

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Connecting COX-2 and Wnt in cancer

Both the cyclooxygenase-2 (COX-2) and Wnt signaling cascades are active in the majority of colorectal cancers. Nevertheless, a direct link between these two key pathways has remained elusive. Recent reports show that one of the bioactive products of COX-2, prostaglandin E_2, activates components of the canonical Wnt signaling system. The findings reviewed below reveal important crosstalk between these pathways, which may provide opportunities for the development of new drugs for treatment and/or prevention of colorectal cancer.

Colorectal cancer is a global concern that accounts for over 50,000 cancer-related deaths each year in the United States alone (Jemal et al., 2005). Colorectal cancer develops following mutations of key oncogenes such as Ras or disruption of tumor suppressor genes such as APC (adenomatous polyposis coli) and p53. The loss of function of DNA repair genes coupled with genomic instability also leads to the development of colorectal cancer. Hereditary predisposition for colorectal polyps and cancer occurs in people with familial adenomatous polyposis (FAP). These patients harbor germline mutations in one allele of the *APC* gene. Upon loss of function of the wild-type *APC* allele, intestinal adenomas develop that eventually progress into colorectal cancer. Interestingly, administration of Celecoxib (Celebrex), which selectively inhibits COX-2, significantly reduces polyp burden in FAP patients. In a murine model for FAP, mice with a germline *APC* mutation (APC^{min}) also develop intestinal polyps. Either treatment with COX-2-selective inhibitors or disruption of the *COX-1* or *COX-2* genes significantly reduces the number and size of intestinal polyps that develop in these mice.

Although there is a temporal association between the loss of APC function and the activity of COX-2 in vivo, there has been little evidence showing a direct connection between these pathways (Shao et al., 2005; Fujino et al., 2002). In a recent report, Castellone et al. identified a direct link between COX-2 and Wnt and have begun to dissect precisely how these signaling cascades are intertwined (Castellone et al., 2005). Using colorectal carcinoma cells in vitro, they show that prostaglandin E_2 (PGE_2) increased the