these factors in the modulation of neoangiogenesis. In addition, it remains to be determined whether these peptides, such as dysplasia-specific homing peptide CSRPRRSEC, play any significant role in wound healing, tissue revascularization after ischemia, or whether this homing is a salient feature of tumors.

Taken together, these findings set forth an enticing hypothesis of a functional vascular heterogeneity that can be studied and used in the future to understand the underlying pathogenesis of tumor neoangiogenic processes. This approach will also provide for new molecular targets for radiographic tumor detection and molecularly directed chemotherapeutic drug delivery and drug sensitivity testing. Given the fidelity in homing of these peptides to their vascular targets, fusion of a specific peptide with an antiangiogenic agent will facilitate delivery of high concentrations of a toxin to disrupt tumor vessels. In light of the unparalleled specificity of phage peptides, there is no doubt that this technology will allow us to identify stage-specific tumor and organ-specific vascular zip codes. This will facilitate identification of therapeutic targets to block tumor angiogenesis and inhibit angiogenic switches with minimal toxicity to other tissue organs, such as those during postnatal development or physiologically regenerative processes.

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Modeling transformation and metastasis in *Drosophila*

Transformation and metastasis are complex, multistep processes. Two recent papers exploit powerful *Drosophila* genetics techniques to explore cooperation between multiple genetic manipulations and to model these processes. In particular, oncogenic Ras is found to collaborate with disruption of cell polarity to trigger massive neoplasia and metastasis. These studies promise further progress in research into the causes of cancer.

Cancer has long been thought of as a multistep process, involving cooperation between several oncogenic events and mutations in tumor suppressors to yield full-blown cellular transformation and metastasis (Hanahan and Weinberg, 2000). *Drosophila* possesses ideal tools to study the clonal effects of multiple genetic manipulations on populations of cells alongside phenotypically normal cells. However, its potential for studying the stepwise nature of the transformation process had so far been largely untapped. This looks set to change with the publication of two complementary reports by the laboratories of Tian Xu and Helena Richardson focusing on cooperation between multiple genetic events to promote neoplastic growth and metastasis (Brumby and Richarson, 2003; Pagliarini and Xu, 2003).

These studies were facilitated by the use of the MARCM technique (Lee and Luo, 2001). Importantly, the MARCM technique allows the generation, in a heterozygous animal, of clones of cells that (1) are homozygous mutant for various mutations, (2) overexpress a transgene(s) of interest, and (3) are labeled positively with a GFP marker. Thus, the MARCM technique is a versatile tool with which the consequences of multiple genetic manipulations upon positively marked cells can be examined in vivo. In both studies, the formation of these clones was targeted to the eye imaginal disc.

A model for metastasis in Drosophila

The Ras protooncogene is activated in about 30% of human cancers (Hanahan and Weinberg, 2000). Ras has been implicated in the metastatic process in mammalian systems, though its precise in vivo contribution is not fully under-

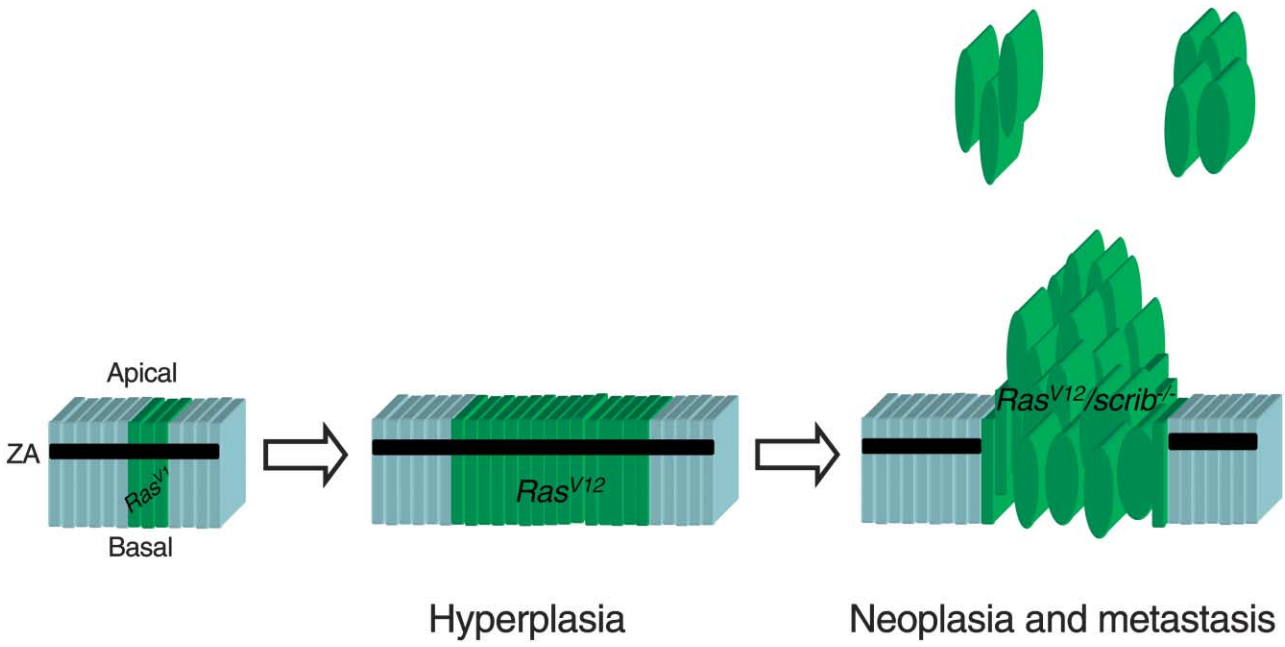

Figure 1. Collaboration between separate genetic events leads to neoplasia and metastasis in *Drosophila* imaginal disc epithelial cells
Expression of activated Ras induces hyperplasia but cells only become metastatic when polarity is perturbed by loss of *scribble*.
ZA = Zonula Adherens.

stood (Bernards and Weinberg, 2002). In *Drosophila*, Ras is required for growth and proliferation, cell survival, as well as the differentiation of many cell types (Baker, 2001). Expression of an activated form of Ras (Ras^{V12}) triggers excessive growth and proliferation, but is insufficient to induce eye imaginal disc cells to become invasive and metastatic. Pagliarini and Xu screened existing mutant collections for mutations that conferred metastatic potential to Ras-expressing cells. Using this approach, they demonstrated that disruption of apico-basal polarity cooperates with activated Ras to induce metastasis. In particular, loss of the cell polarity gene *scribble* (*scrib*) in Ras-expressing cells disrupted the epithelial structure of the eye imaginal disc and led to progressive invasion of these cells into neighboring structures (particularly the ventral nerve chord). Excitingly, these cells could form foci at remote sites within the larval body (see Figure 1).

For Brumby and Richardson, the starting point was *scrib*, which, they show, can collaborate with either Ras^{V12} or an activated form of Notch ($Notch^{ACT}$) to promote neoplastic growth. Their study focused on the mechanism of this uncontrolled neoplastic growth, rather than tissue invasion and formation of secondary foci.

***Cell polarity and tumor suppression in* Drosophila**

Early attempts at using *Drosophila* as a model for cancer yielded a series of genes which, when homozygous, caused massive proliferation of imaginal discs (Humbert et al., 2003). For two of these early tumor suppressors, *lethal(2)giant larvae* (*lgl*) and *discs large* (*dlg*), as well as the more recently identified *scrib*, this excessive growth is accompanied by a loss of epithelial apico-basal polarity. Scrib, Lgl, and Dlg function together to establish basolateral domain identity by antagonizing the function of another group of proteins, the Bazooka (Baz)/DmPAR6/DaPKC (*Drosophila* atypical PKC) complex. The Baz complex sets the position of the adherens junctions and recruits the Crumbs (Crb)/Stardust (Sdt) complex apically to counter the activity of Scrib and its partners (see Humbert et al., 2003; Johnson and Wodarz, 2003). The exact biochemical mechanisms involved have not yet been elucidated. However, these genes are certainly relevant to mammalian polarity since, for example, human *scribble* can rescue the mutant phenotype of its fly counterpart (Dow et al., 2003).

Pagliarini and Xu suggest that loss of cell polarity in general, rather than specific disruption of the Scrib/Lgl/Dlg group, is responsible for cooperation with Ras since mutations in *baz* or *sdt* also induced invasiveness in cooperation with Ras.

Mechanistic aspects of cooperation

In *scrib* homozygous mutants, cells lose apico-basal polarity, overproliferate, and imaginal tissues form large amorphous masses that tend to fuse with other tissues (Humbert et al., 2003). However, in a clonal situation, which mimics the clonal nature of cancer where mutant cells are surrounded by phenotypically wild-type cells, the outcome is quite different. When clones of cells mutant for *scrib* are created in heterozygous animals using mitotic recombination, mutant cells do indeed proliferate excessively, but these excess cells are eliminated by JNK-dependent apoptosis (Brumby and Richarson, 2003). Thus, *scrib* clones fail to overgrow in the presence of wild-type cells (Brumby and Richarson, 2003; Pagliarini and Xu, 2003).

A possible explanation for the cooperation between *Ras* and *scrib* could be

through Ras' ability to promote growth, proliferation, and cell survival, which has been well documented both in *Drosophila* and vertebrates. However, combined overexpression of E2F1/Dp, which promotes cell cycle entry, with the caspase inhibitor p35 failed to trigger neoplasia and invasion in *scrib* mutant cells (Brumby and Richarson, 2003; Pagliarini and Xu, 2003). In addition, mutations in the *warts*/*lats* tumor suppressor, which potently triggers growth and proliferation and blocks apoptosis, do not make *scrib* cells metastasize (Pagliarini and Xu, 2003). Thus, neoplasia and metastasis as a result of loss of polarity depends on specific aspects of Ras signaling, rather than a general ability to induce division and block death. Brumby and Richardson show that activation of one branch of Ras downstream signaling, the Raf/MAPK (mitogen activated protein kinase) pathway, is sufficient to trigger neoplasia in *scrib* mutant cells, whereas other Ras effectors, such as PI3K (phosphoinositide 3-kinase) or Ral, have no effect. The mechanism underlying the specific requirement for Ras activation in triggering neoplasia in combination with *scrib* mutations will be fascinating to study. Ras overexpression is known to modify the adhesive properties of cells in clones in vivo, which may be part of the cooperation mechanism (Prober and Edgar, 2002).

The case of activated Notch is a little more puzzling. Ectopic Notch activation has clearly been shown to trigger uncontrolled growth of imaginal tissues (Go et al., 1998). However, Notch has been implicated in induction of (rather than protection from) apoptosis. Brumby and Richardson suggest that Notch's ability to cooperate with *scrib* may depend on *Ras* function, though the mechanism is unclear. It will be interesting to further probe this question.

Does this represent a bona fide model for mammalian metastasis? Clearly, there are differences. For example, the fly tracheal system (which brings oxygen directly to the various organs) is quite different from the vertebrate circulatory system. Thus extra- and intravasation, both important aspects of the metastatic process that allow dissemination of tumor cells to remote sites, might not be relevant to the fly model. However, *scrib* mutant Ras^{V12}-expressing cells ($Ras^{V12}/scrib^{-/-}$ cells) were observed in tracheal branches, though the mechanism of entry is not yet known (Pagliarini and Xu, 2003).

Pagliarini and Xu present two additional lines of evidence that draw strong parallels between their *Drosophila* metastasis model and mammalian metastasis. First, loss of E-Cadherin expression is a feature of many invasive cancers, and expression of *Drosophila* E-Cadherin is reduced in $Ras^{V12}/scrib^{-/-}$ cells. Moreover, expression of E-Cadherin efficiently blocked metastasis of $Ras^{V12}/scrib^{-/-}$ cells. Second, the ability of tumor cells to degrade the extracellular matrix (ECM) contributes to invasion and metastasis (Hanahan and Weinberg, 2000). Using a *collagen-GFP* protein trap and an anti-Laminin antibody, Pagliarini and Xu show that $Ras^{V12}/scrib^{-/-}$ cells are able to disrupt the ECM. Thus, several key aspects of mammalian metastasis are recapitulated in the *Drosophila* model.

What next?

This work opens many exciting avenues for future studies. How does cell polarity affect neoplasia and metastasis? Loss of cell adhesion is clearly a feature of mammalian cancer (Hanahan and Weinberg, 2000). The relative contributions to transformation and metastasis of processes such as loss of contact inhibition or modifications in cell signaling resulting from polarity loss can readily be tackled in the *Drosophila* model.

Another interesting aspect of this work concerns the nature of JNK-dependent apoptosis that limits growth of *scrib* mutant tissues (Brumby and Richarson, 2003). This requires the presence of neighboring wild-type cells and is reminiscent of the JNK-dependent apoptosis elicited by cell competition or disruption of tissue patterning cues (Adachi-Yamada and O'Connor, 2002; Moreno et al., 2002). One possible explanation is that loss of polarity perturbs the transmission of morphogenetic signals, thus triggering apoptosis of aberrant cells. In addition, little is currently known about *scrib*'s role in repressing proliferation, which warrants further investigation.

The two groups propose complementary approaches to follow up on their exciting results: a search for mutations in new metastasis suppressors that confer invasiveness to Ras-expressing cells as well as a search for "oncogenic mutations" that cooperate with *scrib* mutations to promote neoplastic growth. The combination of these screens should provide exciting insights into the complexities of cancer biology.

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