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Minireview

Drosophila as an emerging model to study metastasis

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Abstract

Metastasis is the primary cause of human cancer-related deaths. Two recent studies describe a system for testing how multiple genetic events synergize to promote neoplastic growth and metastasis in *Drosophila*, paving the way for systematic approaches to understanding metastasis using the powerful tools of *Drosophila* genetics.

Modeling metastasis in *Drosophila*

Cancer can be thought of as a genetic disease caused by the accumulation of multiple genetic or epigenetic lesions in tumor-suppressor genes and oncogenes; the resulting processes culminate in cell proliferation, survival and metastasis (colonization of distant sites by tumor cells) [1]. But depending on the sequence in which cells accumulate genetic lesions, the ensuing tumor progression and metastasis are highly variable, even among tumors of the same type [1]. Furthermore, only a small fraction of tumor cells achieve metastatic competence, suggesting that the combination of required events is acquired only rarely. Our current understanding of the molecular processes leading to metastasis is largely derived from studies of cancer cell lines *in vitro*, xenografts of human tumors, and a limited number of transgenic or knockout mouse models [2,3]. These systems may not reflect the normal processes involved in tumorigenesis or, as in case of murine models, may be too cumbersome to be used for analyzing multiple genetic interactions. A major challenge in cancer research is, therefore, to develop model systems that allow efficient analysis of the combinations of genetic events that trigger the initiation of metastasis during tumor development *in vivo*.

In order to study metastasis *in vivo*, it would be ideal to use a model organism in which multiple genetic lesions could be introduced in a controlled way into subpopulations of cells, which can then be analyzed for proliferation, cell migration, invasion and metastasis. The fruit fly *Drosophila* researchers have an arsenal of sophisticated genetic manipulation techniques that have proven invaluable for dissecting the signaling pathways that affect cell specification, differentiation and growth [4-7]. Furthermore, *Drosophila* is highly amenable to genetic screens for previously unidentified components of signaling pathways. Knowledge gained using *Drosophila* is often applicable to human biology, because pathways controlling virtually all cell-biological processes are highly conserved between flies and humans [8]. Two papers, by the Richardson and Xu groups [9,10], now demonstrate the use of *Drosophila* to investigate the combinations of genetic aberrations that trigger metastasis.

The key to studying how combinations of genetic alterations cause metastasis in *Drosophila* was the generation of clones of cells that carried multiple genetic manipulations such as mutations in tumor-suppressor genes and overexpression of oncogenes [9,10]. In addition, the manipulated cells also expressed green fluorescent protein (GFP), which allowed their detection and analysis of their proliferation, migration and invasive behavior *in vivo* in the context of unmanipulated cells. Such GFP-marked clones were generated by using a powerful genetic technique called 'mosaic analysis with a repressible cell marker' (MARCM), which produces clones of cells that are mutant for a gene of interest and that

simultaneously overexpress one or more genes, such as oncogenes and GFP [11]. Using MARCM clones, the Richardson [9] and Xu [10] groups found that a specific combination of defects, namely disruption of apical-basal cell polarity together with the expression of an activated version of the oncogene *Ras*, results in excessive cell proliferation and metastasis.

Genetic lesions that synergize to give metastasis

Both groups [9,10] found that overexpression of oncogenic *Ras* (the Ras^{V12} variant) in clones mutant for the tumor-suppressor gene *scribble (scrib)* resulted in excessive proliferation and metastasis [9,10]. Scrib is an adaptor protein containing multiple PDZ domains (which enable binding to a variety of other proteins); it is localized to basolateral membranes of epithelial cells [12]. The Xu group [10] tested whether mutations in tumor-suppressor genes - such as *scrib* or *warts*, which encodes a protein kinase of the DMPK family - synergize with oncogenic Ras to induce metastasis, whereas the Richardson group [9] examined the effects of activation of various signaling pathways on the growth and survival of *scrib* mutant cells. To do so, both groups induced MARCM clones specifically in the cephalic complexes of *Drosophila* larvae (the eye imaginal discs, which are epithelial sacs that give rise to adult eyes, and the optic lobes of the brain) using a tissue-specific driver containing the promoter of the *eyeless* transcription-factor gene coupled to the Flp recombinase gene (*eyFLP*). *eyFlp* induces mitotic recombination, producing clones of homozygous mutant cells [13]. Mutant cell clones were then analyzed for cell proliferation and for invasion of distant larval tissues. $Ras^{V12}scrib^{-}$ mutant cells overproliferate and form secondary tumors in the ventral nerve cord, imaginal tissues and tracheal branches in the mutant animals [9,10].

This effect is striking because neither loss of *scrib* nor overexpression of Ras^{V12} individually causes invasive behavior [9,10,14]. Loss of *scrib* in homozygous mutant animals results in loss of epithelial (apical-basal) polarity and causes neoplastic overgrowths of imaginal tissues [12,15]. Overexpression of oncogenic Ras alone results in hyperplastic overgrowth of imaginal tissues [14]. Thus, loss of *scrib* or activation of oncogenic Ras can induce only excessive proliferation, but not metastasis, in imaginal tissues. In addition, deletion of *warts* does not phenocopy *scrib* deletion in cells overexpressing Ras^{V12} [10]. The synthetic effect of loss of *scrib* function with Ras activation thus represents a specific combination of genetic changes that together induce metastasis.

Pagliarini and Xu [10] asked whether the metastasis observed in $Ras^{V12}scrib^{-}$ cells exhibits features of human metastatic tumors. Normally, the integrity of epithelial tissues is maintained by adhesive cell-cell interactions [16]. Defects in tissue integrity allow tumor cells to acquire motility and become metastatic; as part of this process, mammalian metastatic tumors often downregulate the cell-adhesion molecule E-cadherin [17]. Similar effects occur in *Drosophila*, in which E-cadherin expression is downregulated in $Ras^{V12}scrib^{-}$ tumors [10]. Downregulation of E-cadherin is important for the metastatic behavior of *Drosophila* tumors, because overexpression of E-cadherin suppresses metastasis in $Ras^{V12}scrib^{-}$ cells. Loss of *E-cadherin* does not mimic the loss of *scrib,* however, because metastasis is not observed upon activation of Ras^{V12} in *E-cadherin* mutants. The loss of *scrib* must therefore have effects in addition to loss of *E-cadherin* to promote metastasis in cells overexpressing oncogenic Ras. Another ability that is acquired by mammalian tumors is degradation of basement membranes. Using an antibody to detect laminin and a collagen IV-GFP fusion protein to detect basement membranes, Pagliarini and Xu [10] found that metastatic $Ras^{V12}scrib^{-}$ tumor cells degrade basement membranes in *Drosophila*. By the criteria of loss of cell adhesion and degradation of basement membranes, therefore, they concluded that these metastatic tumors mimic critical features of metastatic mammalian tumors [10].

The *scrib* gene has a pivotal role in establishing cell polarity in association with two other *Drosophila* neoplastic tumor-suppressor genes, *lethal(2) giant larvae* and *discs large* [15,18]. The *lethal(2) giant larvae* gene encodes a protein that contains WD40 repeats (predicted to be involved in protein-protein interactions) and *discs large* encodes a member of the membrane-associated guanylate kinase (MAGUK) family that contains multiple PDZ domains known to be involved in targeting signaling proteins to the cell membrane. Pagliarini and Xu [10] therefore asked whether the metastatic behavior of $Ras^{V12}scrib^{-}$ cells is due to loss of cell polarity. They found that *lethal(2) giant larvae*, *discs large* and other cell-polarity genes such as *bazooka* and *stardust*, both of which encode multi-PDZ domain-containing proteins, as well as *cdc42*, encoding a small GTPase, which individually do not affect growth, also impart metastatic potential to cells overexpressing Ras^{V12}. Thus, loss of cell polarity in general, not just through loss of *scrib*, collaborates with oncogenic Ras to promote metastasis [9,10]. Defects in cell polarity result in an epithelial-to-mesenchymal transition, disruption of intercellular junctions and loss of cell adhesion; these defects may be important for the progression of cells from neoplastic (tumorigenic) to metastatic behavior.

A new role for Ras in metastasis

Why do mutations in *scrib* synergize so strongly with activation of Ras? As in vertebrates, Ras in *Drosophila* promotes cell survival, growth and proliferation [19]. If the effects of Ras function through these processes, then overexpression or overactivation of the downstream effectors involved in cell survival, growth and proliferation in *scrib* mutant cells should mimic the effects of Ras^{V12}. Overexpression of an

activated version of the Ras effector protein Raf mimics the effects of Ras overexpression in *scrib* mutant cells, indicating that Ras mediates its oncogenic effects by signaling through Raf [9]. Clones of cells mutant for *scrib* alone survive poorly [9,10], and Ras may thus collaborate with *scrib* to promote their survival. The poor survival of *scrib* mutant cells depends on the presence of neighboring wild-type cells, and *scrib* mutant cells survive if the entire tissue is mutant [12]. It seems, therefore, that *scrib* mutant cells are not destined to die because of intrinsic defects in their cell physiology, but rather they are actively killed by a process requiring interactions between *scrib* mutant and neighboring wild-type cells, a process referred to as cell competition [20]. The nature of the death-inducing signal is not known, but the elimination of *scrib* mutant cells requires activation of signaling through the Jun N-terminal kinase (JNK) pathway, as is the case for other manipulations that trigger cell competition [21-23]. Thus, blocking JNK signaling in *scrib* mutant cell clones rescues them from apoptosis [9]. These cells still do not metastasize, however; and Ras^{V12} must therefore have effects, in addition to promoting survival of *scrib* mutant cells, that impart metastatic potential.

To test whether promotion of cell proliferation and cell growth can together mimic the effects of activated Ras, the two groups overexpressed the transcription factor E2F together with its binding partner Dp, to promote cell proliferation, and the phosphatidylinositol 3-kinase Akt or the transcription factor Myc, to promote cell growth, in *scrib* mutant cells. Overexpression of any of these effectors alone did not mimic the effects of Ras^{V12}, however. Moreover, coexpression of E2F, Akt and the apoptosis inhibitor p35 together also did not mimic the effects of Ras^{V12}. Thus, the combination of blocking cell death (with p35), promoting cell proliferation (E2F/Dp) and promoting cell growth (Akt) does not phenocopy the effects of Ras activation [9,10]. Ras therefore mediates its oncogenic effects through other (unknown) downstream effectors [9,10].

The Richardson group [9] tested whether other signaling pathways can cause *scrib* mutant cells to metastasize. They found that activation of the signaling pathways initiated by any of the extracellular ligands Decapentaplegic, Hedgehog and Wingless did not show a strong effect on *scrib* mutant cells [9]. In contrast, the activation of the Notch receptor signaling pathway had similar effects on *scrib* mutant cells to the coexpression of oncogenic Ras, but it remains to be determined whether Notch signals through Ras or independently [9].

Advantages of *Drosophila*

The MARCM system can mimic the clonal nature of mammalian cancer, because it allows simultaneous manipulation of multiple genes in small populations of cells. The metastasis models described in the two recent papers [9,10] can now be used to perform systematic screens for secondary mutations that modify (enhance or suppress) the metastatic phenotypes. It is therefore now possible to do genome-wide screens for mutations that promote growth and metastasis in combination with other oncogenes and tumor-suppressor genes. Even though not all aspects of human cancer and metastasis may have parallels in flies, we can expect many exciting new discoveries to be made using flies as a model to study metastasis.

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