Why should we care about fly tumors?
The case of JAK-STAT and EGFR cooperation in oncogenesis

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Drosophila is proving to be a valuable model for studying aggressive tumors induced by the combined activation of EGFR and JAK-STAT signaling. Here we summarize some of the most recent data showing that tissue damage and the modulation of common pathway regulators are at the heart tumor progression and metastasis.

Cancer is a complex disease in which a variety of signals contribute to tumor generation and progression. This complexity is increased if we consider how the interaction of the tumor with its cellular environment contributes to the regression or expansion and aggressiveness of the lesion.

Remarkable advances have been made to treat different types of cancer. However, treatments based on the true elimination of tumor cells at early stages of cancer progression would be crucial to improve the yield of successful therapies. Early factors that trigger tumor development can be studied in Drosophila, a genetically tractable model organism.

Given the large evolutionary distances separating Drosophila and humans, flies may seem a bad choice as model system for cancer research. However, experiments done in the last few years proved that tumors can be readily induced in Drosophila by expressing or mutating the same genes involved in human tumors.

More than 68% of the genes involved in human cancer are conserved in Drosophila, among others the EGFR and JAK-STAT pathways. The potent Drosophila tool-kit developed to study the involvement of these pathways in normal development is now being used to find out how these genes control cell proliferation and how in some conditions their abnormal regulation induces aggressive tumors. The unparalleled capacity for genetic manipulation in Drosophila permits activating or repressing any gene combination in labeled cells at particular regions. This allows studying how these genes induce over proliferation, metastasis and how the tumorous tissue displaces the normal cells. These techniques also allow the systematic activation of other genes in the tumor in order to identify new molecules that suppress tumor growth and therefore could become drug targets to treat tumors in humans. In this commentary we want to discuss recent developments describing how simultaneous misregulation of the EGFR and the JAK-STAT pathways in Drosophila epithelial cells induce carcinomas, how this carcinomas interact with the cellular microenvironment and how competition between normal and tumorous cells leads to the regression or expansion of the tumor.

It had been observed that activating the EGFR pathway in flies, either by ectopically expressing the receptor or by activating its downstream target Ras (with the constitutively activated RasV12 mutation), can cause benign epithelial tumors. In these tumors the epithelial tissues over proliferate without the cells losing their epithelial character, with clear apical localization of E-cadherin and maintenance of the cell polarity (Fig. 1A and B). Later work showed that mutation of secondary genes in cells with overactive EGFR
pathway could cause over-proliferation and induce aggressive carcinomas that metastasize.\textsuperscript{2,4} Especially interesting was the interaction found between cell polarity mutations and EGFR pathway activated cells. As mutant flies develop epithelial tissue overgrowth that eventually kills the animal (explaining the telling names of these mutations). Surprisingly, instead of forming a tumor, small groups of cells carrying mutations of these epithelial polarity genes are eliminated from the tissue by competition with the surrounding normal cells.

A very different situation arises when mutations on \textit{scrib}, \textit{dlg} or \textit{lgl} occur in Ras activated cells. In this case the double mutant cells survive the competition and become metastatic\textsuperscript{2,4} proving that, as in humans, tumor aggressiveness results from more than one lesion. To investigate why this gene combination causes such invasiveness, the fly tumors were studied by microarray analysis and it was found that the three \textit{upd} ligands of the Drosophila JAK-STAT pathway where highly upregulated.\textsuperscript{7} Studying the functional requirement of JAK-STAT signaling in these tumors was facilitated by the simplicity of the Drosophila pathway that consists of a single receptor (\textit{dome}), a single JAK kinase and a single STAT transcription factor (\textit{Stat92E}).\textsuperscript{8,9} This allowed to find that the tumors caused by activated \textit{RasV12} and \textit{scrib}\textsuperscript{−} mutants were suppressed by expression of a dominant negative JAK-STAT receptor or by mutation of the \textit{Stat92E} gene\textsuperscript{7} suggesting that the EGFR tumor becomes much more aggressive by the simultaneous activation of the JAK-STAT pathway. This point was confirmed by the observation that expressing the \textit{upd} ligands in Ras activated cells results in large metastatic tumors. It was also found that the activation of the \textit{upd} ligands in \textit{scrib}\textsuperscript{−} mutant cells with affected polarity was mediated by the JNK pathway activation. Indeed, mutating the Jun kinase gene \textit{basket} in \textit{RasV12 scrib}\textsuperscript{−} mutant cells suppressed the metastasis. Interestingly, the same mutation is unable to suppress tumors caused by ectopic \textit{upd} ligand in \textit{RasV12} cells, indicating that the loss of cell polarity activates the JNK pathway that in turn activates JAK-STAT (Fig. 1C). Thus, the co-activation of JAK-STAT and the EGFR pathway is ultimately responsible for the aggressive carcinoma as blocking JAK-STAT activation at any level can ameliorate the tumor progression in \textit{RasV12} cells.

The \textit{Scribble} (\textit{scrib}), \textit{lethal (2) giant larvae} (\textit{lgl}) and \textit{discs-large} (\textit{dlg}) genes encode proteins that form a complex on the basolateral membrane that is necessary to maintain normal epithelial cell polarity. These genes are tumor suppressors\textsuperscript{5,6} as mutant flies develop epithelial tissue overgrowth that eventually kills the animal (explaining the telling names of these mutations). Surprisingly, instead of forming a tumor, small groups of cells carrying mutations of these epithelial polarity genes are eliminated from the tissue by competition with the surrounding normal cells.

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**Figure 1.** The combined action of EGFR and JAK-STAT signaling results in tumor progression.

(A) Epithelial growth in tissues with normal apico-basal cell polarity is controlled by the balanced activity of EGFR, JAK-STAT and other signaling pathways. Negative regulators such as Socs36E control pathway activity by modulating signaling output. (B) When EGFR is over activated (shown in bold), the epithelium proliferates excessively without necessarily causing metastasis. (C) Over proliferation and metastasis are promoted by the combined misregulation of EGFR and high JAK-STAT expression when the signaling balance is broken by the downregulation of Socs36E by bantam miRNA expression or by JNK mediated JAK-STAT activation.
Although in the above-described experiments the loss of polarity and the activation of the EGFR pathway both happen in the tumor cells, metastatic tumors also appear if the polarity defect is induced in the neighbor cells to those where Ras is active. This implies a non-clonal origin of the tumor with cell interactions inducing the metastatic behavior. In this last case the scrib cells were instrumental for starting the aggressive tumor by inducing the JNK pathway. JNK activation from scrib mutant cells can spread to the neighboring Ras cells that will activate JAK-STAT signaling. Although the scrib mutant cells eventually disappear due to cell competition, they activated the invasive cocktail of factors that allow the activated Ras cells to become metastatic.

In their recent publication Herranz and collaborators explore how this EGFR JAK-STAT oncogenic activation cocktail may act. In this work the authors were investigating Drosophila genes that would increase the proliferation potential of EGFR overexpressing cells. They found that co-expression of EGFR with the bantam microRNA (miRNA), which has been shown to be involved in growth control, results in massive epithelial overgrowth accompanied by a loss of epithelial polarity that is not observed when the genes are expressed independently. The authors show that expression of EGFR results in activation of Socs36E in the epithelium, and that bantam miRNA exacerbates the EGFR overexpression consequences through the downregulation of Socs36E expression. A similar effect to bantam expression is achieved if the EGFR is coexpressed with a Socs36E RNAi. Thus the activation of Socs36E establishes a brake to EGFR overproliferation and metastasis that is lifted by bantam expression (Fig. 1C). In Drosophila, Socs36E is a direct transcriptional target of JAK-STAT and Socs36E has been shown to downregulate EGFR and JAK-STAT signaling. Herranz and collaborators show that bantam expression or Socs36E downregulation lead to a strong activation of JAK-STAT in EGFR overexpressing cells. Downregulation of the JAK-STAT receptor dome or the Stat92E transcription factor in these metastatic cells results in tumor size normalization, indicating that the EGFR JAK-STAT cocktail is responsible for the aggressive tumorous overgrowth.

Why do EGFR JAK-STAT metastatic tumors displace the normal tissues? A possible explanation comes from the process of cell competition where it has been shown that cells that proliferate faster displace normal neighboring cells that proliferate less. Cell competition has been observed to occur among cells with different levels of ribosomal proteins, Myc or Yorkie, but a recent paper provides data suggesting that cells with higher activation of JAK-STAT pathway are more competitive than cells with lower levels. The authors showed this in two ways: First, they induced simultaneously a clone of homozygous Stat92E mutant cells and a neighboring clone of wild-type cells and observed that the Stat92E mutant clone gave rise to less daughter cells than the wild type. This was not due to an intrinsic defect in the Stat92E mutant cells, as using established systems that increase cell competitiveness during development (for example when they produce more ribosomal proteins than the wild-type cells) allowed the Stat92E clone to proliferate normally. Second, they showed that activation of the JAK kinase has the complementary effect, making cells proliferate more efficiently and out-compete their wild-type neighbors. Mechanistically, cell competition is due to the JAK activated cells inducing apoptosis on the neighboring wild-type cells, allowing the first to occupy a larger fraction of the tissue. Despite the overproliferation the final tissue is not overly aberrant in shape, indicating that the competitive advantage is checked by the normal developmental and homeostatic controls. Although it is not yet clear how the activation of the JAK-STAT pathway transforms cells into super competitors, it is an interesting avenue of research that may help understanding how the EGFR JAK-STAT cocktail induces cells to become highly invasive.

Another recent paper demonstrates that cells require Stat for competitive fitness to eliminate neighboring scrib clones. scrib clones are eliminated by cell competition when surrounded by normal cells. However, when scrib cells are abutting Stat92E cells, the elimination does not occur and instead the scrib clone overgrows. Thus, these findings show that Stat protects the tissue from invasiveness.

Are these observations relevant for human metastasis? The conservation of the pathways and regulatory elements suggest they are, and some observations indicate there is a deeper conservation in the metastatic processes that goes further than those unveiled by sequence conservation. Although Drosophila Socs36E has got in SOCS5 a human ortholog, there is no homology between bantam miRNA and any human miRNA. SOCS5 behaves as a tumor suppressor in an EGFR/RAS dependent cell transformation assay. Recent work shows that a similar genetic interaction to that of bantam and Socs36E, occurs in vertebrate tumor endothelial co-cultures with SOCS5 and the miR-9 miRNA. mir-9 is secreted from melanoma cells but not from normal skin melanocytes and is taken up by endothelial cells where SOCS5 is downregulated and as a result STAT1 becomes activated. This activation induces the migration of endothelial cells toward the tumor. Downregulation of JAK activation reverts the endothelial growth. This mechanism of regulation reveals a functional similarity between Drosophila and humans even though there are clear sequence differences between the miRNAs involved and even between the target tissues.

These studies and the functional similarities they uncover should make us aware that, as humans, we should keep a selfish interest on the developments in fly tumor research.

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