Breaking Down Neighbors to Fuel Tumorigenesis

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Autophagy supports cell growth and survival autonomously by recycling intracellular proteins and/or organelles. Reporting in *Nature*, Katheder and colleagues (2017) find that tumors trigger non-autonomous autophagy in neighboring cells and distant organs, thus fueling tumor growth and metastasis. This opens new avenues for understanding and manipulating cancers through cell-cell communication.

Tumorigenesis entails the complex interplay between tumors and their microenvironment (Enomoto et al., 2015). Autophagy, the controlled breakdown and recycling of intracellular proteins and/or organelles, is implicated in regulating tumorigenesis (Amaravadi et al., 2016). While autophagy's role is acknowledged to be complex, current dogma generally emphasizes an autonomous, tumor-promoting role by enabling cancer cell survival when faced with nutrient deprivation and metabolic stress (Amaravadi et al., 2016). For example, Ras-activated malignant tumors were autonomously dependent on autophagy in a *Drosophila* model (Pérez et al., 2015). Reporting in *Nature*, Katheder and colleagues (2017) have discovered that tumors in the same *Drosophila* model predominantly trigger non-autonomous autophagy (NAA) in neighboring cells and distant organs to promote tumor growth and metastasis.

Katheder and colleagues (2017) utilize a model where oncogenic Ras (Ras^{V12}) is overexpressed in clones of cells with aberrant apicobasal polarity (*scribble;* $scrib^{-/-}$) in eye-antennal imaginal discs, the epithelial precursors to adult fly organs. Unlike benign Ras^{V12} tumors, Ras^{V12}, $scrib^{-/-}$ tumors metastasize (Pagliarini and Xu, 2003) and induce systemic organ wasting through insulin inhibition (Figueroa-Clarevega and Bilder, 2015). Strikingly, the authors observed that in malignant Ras^{V12}, $scrib^{-/-}$ tumors, autophagy was most strongly upregulated in surrounding, wild-type epithelial cells.

To test the functional role of NAA, the authors pharmacologically inhibited autophagy with chloroquine, which halved tumor size and partially suppressed metastasis. However, chloroquine can suppress neoplasia through non-autophagic pathways (Amaravadi et al., 2016). To more precisely address autophagy's role in Ras^{V12}, $scrib^{-/-}$ tumorigenesis, Katheder et al. (2017) specifically removed key autophagy genes within the tumor, within the surrounding tissue, within both, or within the entire larva. While blocking autophagy in the tumor partially reduced tumor volume, it did not prevent metastasis. In contrast, removing autophagy function only in neighboring, wild-type epithelial cells more strongly suppressed tumor growth and also limited metastasis. Strikingly, blocking autophagy throughout the entire larva resulted in the strongest suppression of tumor growth and metastasis, hinting at systemic effects. Indeed, even though tumors were induced in eye-antennal discs, autophagy was systemically upregulated in gut, fatbody, and muscle tissues (Figure 1A). Moreover, in autophagy-compromised *atg13* (*autophagy-related 13*) mutant larvae, re-expression of Atg13 specifically in eye discs only partially rescued tumor growth, further supporting that tumorigenic signals also arise from distal autophagy. Elegant transplantation experiments provided the strongest evidence for systemic autophagy abetting tumorigenesis: autophagy-deficient, dormant Ras^{V12}, $atg13^{-/-}$, $scrib^{-/-}$ tumors could reinitiate growth if transplanted into autophagy-competent, but not autophagy-deficient, host adults. Thus, NAA operates both locally and systemically to support tumor growth and invasion (Figure 1).

How do Ras^{V12}, $scrib^{-/-}$ tumors provoke elevated autophagy in neighboring, wild-type cells? Ras^{V12}, $scrib^{-/-}$ tumors strongly deregulate JNK (c-Jun N-terminal kinase), Hippo, and JAK-STAT signaling pathways (Atkins et al., 2016). Katheder et al. (2017) found that all three were autonomously required within tumors to trigger local NAA (Figure 1B). Importantly, loss of NAA caused by inhibiting any of these pathways was not trivially due to impaired tumor growth, as smaller, PI3K-disrupted tumors still activated NAA. Interestingly, activation of JAK-STAT in plain Ras^{V12} cells by overexpressing the secreted ligand Upd/IL-6 could trigger NAA. However, mutating *stat* only in wild-type neighbors of Ras^{V12}, $scrib^{-/-}$ tumors did not abolish NAA, indicating that JAK-STAT signaling is required specifically in tumors to induce NAA. The authors also found that reactive oxygen species (ROS) were strongly upregulated in Ras^{V12}, $scrib^{-/-}$ tumors, though a functional requirement for ROS in NAA was not demonstrated. Moreover, overexpressing Yorkie (Yki, a YAP homolog) triggered NAA, but not ROS, further questioning the role of ROS in NAA. Thus, autocrine Upd/IL-6 and autonomous JNK/Yki/STAT signaling in tumors are essential for the induction of local NAA, but the signals propagating from tumors to neighboring cells remain unclear (Figure 1B).

Although Katheder and colleagues (2017) identified key pathways regulating local NAA, the etiology of systemic autophagy is likely distinct, as impairing JNK signaling within tumors abrogated local NAA yet had no effect on systemic autophagy in muscles or fatbody. A prime candidate for inducing systemic autophagy was Impl2, a tumor-secreted insulin antagonist that induces cachexia-like wasting in Ras^{V12}, $scrib^{-/-}$ larvae (Figueroa-Clarevega and Bilder, 2015). However, the authors found no role for Impl2 in NAA, and thus the mechanisms of systemic autophagy induction remain mysterious.

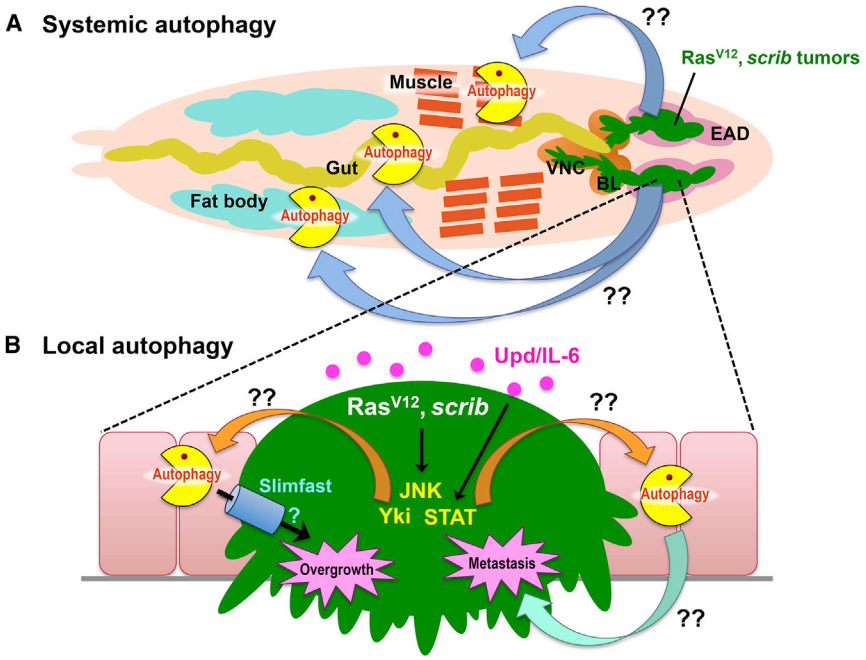

Figure 1. Tumors Trigger Non-autonomous Autophagy Both Locally and Systemically
(A) *Drosophila* larvae harboring Ras^{V12}, *scrib*$^{-/-}$ tumors (green) in eye-antennal discs (EAD) undergo systemic autophagy. Tumors can metastasize from brain lobes (BL) and/or EAD into ventral nerve cord (VNC). (B) Local non-autonomous autophagy (NAA) fuels Ras^{V12}, *scrib*$^{-/-}$ growth and metastasis. JNK, Yki, and autocrine JAK-STAT signaling activated by Upd/IL-6 trigger NAA. NAA may contribute to tumor growth by providing nutrients to tumors, including through the amino acid transporter Slimfast.

How does NAA fuel Ras^{V12}, *scrib*$^{-/-}$ tumorigenesis? An intuitive explanation is that autophagy in neighboring cells releases nutrients that are pilfered by metabolically stressed tumors (Martinez-Outschoorn et al., 2017). Three lines of evidence from Katheder et al. (2017) support this hypothesis. First, blocking the amino acid transporter *slimfast* in tumors strongly truncated tumor growth (Figure 1B). Second, Ras^{V12}, *scrib*$^{-/-}$ tumors certainly appear "stressed," with upregulated ROS, glucose consumption, and morphologically aberrant mitochondria. Third, blocking NAA impaired tumor cell cycling and proliferation but did not alter apoptosis. Identification of the NAA-generated catabolites that are essential for tumorigenesis would help to directly prove this hypothesis.

These data lay exciting groundwork for exploring NAA in tumorigenesis (Figure 1), but many tantalizing questions remain unanswered. The nature of local and systemic NAA remains uncharacterized, and it is unknown which catabolites and/or signals functionally feed the tumor. On the signal-sending side, what downstream of JNK/Yki/STAT is transmitted from tumors to wild-type cells to drive local and systemic autophagy? This could involve cell-to-cell propagation of JNK/Yki activity, as seen in Src-activated cell clones or in wound healing and regeneration (Enomoto et al., 2015). Once NAA is induced, how does it facilitate metastasis? Autophagy autonomously controls focal-adhesion turnover to impact cell migration (Amaravadi et al., 2016), but this does not readily explain a non-autonomous role for autophagy in cell invasion. Finally, is the mechanism of NAA more broadly employed in other tumor models? Katheder et al. (2017) found that Yki-induced tumors trigger but do not depend on NAA for growth, suggesting that tumor-induced NAA is not always required for tumors to grow. Additionally, Myc-overexpressing "supercompetitor" cells were found to undergo increased glycolysis (de la Cova et al., 2014), similar to Ras^{V12}, *scrib*$^{-/-}$ tumors. These cells are dependent on p53 for maintaining their high metabolic flux and "winner" status during cell competition, which is intriguing given the wealth of data linking p53 to autophagy (Amaravadi et al., 2016). These observations raise the possibility that localized NAA is triggered by and/or influences cell competition.

Katheder and colleagues (2017) have provided compelling genetic evidence for NAA fomenting tumorigenesis. A similar phenomenon in mammals was recently uncovered where pancreatic ductal adenocarcinoma triggered localized NAA in pancreatic stellate cells, thereby generating alanine and fueling tumor growth (Sousa et al., 2016). These studies emphasize the need to continue improving the specificity and potency of current autophagy inhibitors for clinical trials (Amaravadi et al., 2016) and suggest that previously ascribed tumor-suppressive properties of autophagy inhibitors should be reevaluated in the context of potential NAA. However, as autophagy also has tumor-suppressive functions and is likely involved in cancer-immune system interactions (Amaravadi et al., 2016), further testing is required to determine which tumors are actually autophagy dependent. Nonetheless, the initial genetic dissection by Katheder and colleagues (2017) paves the way for a better understanding of complex, non-autonomous tumor-promoting programs.

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