confinement in scenarios such as densely packed tumors.

This elegant study from Matthews et al. (2020) opens several exciting avenues for future investigation. A crucial next step will be to take these cell-culture-based findings into whole tissues to explore the role of Ras^{V12}-mediated cell rounding and stiffening. It will be particularly useful to investigate this process within both *in vivo* and *ex vivo* models of primary tumor and cancerous tissues at different stages of disease progression. This would help address whether the proposed pathway is dependent on cancer stage, since a recurring observation throughout the study is that short-term activation of Ras signaling may be enough to facilitate mitotic rounding in confined conditions. To that end, it is also noteworthy to consider whether the presence of other prevalent cancer mutations in addition to Ras mutations affects this pathway. Among these, mutations in proto-oncogenes such as Ect2 and Moesin may be of particular interest, since the downstream products of these genes are associated with enhanced mitotic rounding (Carreno et al., 2008; Kunda et al., 2008; Matthews et al., 2012). Moreover, in tissues, where cell-cell connections are key to structural integrity, it will also be crucial to understand how Ras-dependent rounding in a mitotic cell is affected by neighboring cells. Furthermore, a variety of mechanical forces, such as stretch, compression, and shear, contribute to cell confinement in a growing tumor. To develop a thorough understanding of how cells adjust their divisions within confinement, it will be important to dissect the extent to which rounding and ultimately cell division are affected under these distinct force regimes.

Preventing growth and proliferation of tumors is a principal strategy in cancer therapy. By investigating cell divisions under confinement, Matthews et al. (2020) shed important light on a fundamental mode of action that cancer cells may utilize to enhance their survival in the complex tumor environment.

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Autophagy Suppresses Breast Cancer Metastasis

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Cancer cells need to acquire specific molecular traits in order to spread to distant organs. In this issue of *Developmental Cell*, Marsh et al. show that autophagy restricts the outgrowth of breast cancer metastases in contrast to its impact on primary tumor progression.

Metastasis, the spread of cancer cells from the original tumor to other parts of the body, is the major cause of cancer-associated death. In order to successfully colonize distant organs, cancer cells must overcome a series of bottlenecks. To this end, they acquire specific molecular traits, many of which are poorly defined, but whose understanding holds therapeutic potential for inhibiting this fatal process. In this issue of *Developmental Cell*, Marsh et al. (2020) reveal that autophagy, a catabolic process that leads to the degradation of cellular components, suppresses the outgrowth of breast cancer lung metastases.

Autophagy is an evolutionary-conserved process that enables cells to mobilize and recycle cellular nutrients. During autophagy, double-membrane-bound vesicles termed autophagosomes engulf cytoplasmic proteins and organelles. Autophagosomes then fuse with lysosomes, leading to the degradation and recycling of the engulfed cargo. Autophagy is tightly controlled and plays particularly important roles during cellular

starvation and stress. The role of autophagy in cancer is complex. Genetic models of cancer initiation have shown that autophagy acts as a tumor suppressor during tumorigenesis (Galluzzi et al., 2015). Conversely, autophagy promotes progression of established tumors (White, 2012), prompting multiple clinical trials to investigate the use of autophagy inhibitors in cancer. Despite these established context-dependent roles in cancer and its targeting in clinical studies, little is known about the impact of autophagy on cancer metastasis.

To investigate the effect of autophagy on breast cancer metastasis, Marsh et al. (2020) derived cell lines from mouse breast tumors engineered to allow for the inducible ablation of autophagy. The authors injected these cells into the systemic circulation of mice and ablated autophagy in these cells by genetically inactivating autophagy genes *Atg5* or *Atg12*. Notably, autophagy-deficient breast cancer cells formed significantly larger metastases containing more proliferative cells relative to autophagy-competent cells. Autophagy-deficient breast cancer cells also exhibited a higher capacity to form metastases from orthotopic primary tumors. These data indicate that, in contrast to its role in primary tumors, autophagy suppresses metastatic outgrowth of disseminated breast cancer cells.

How does autophagy suppress metastatic outgrowth? To answer this question, the authors assessed how ablation of autophagy impacted the transcriptome of metastatic cells. Autophagy-deficient cells exhibited a transcriptional shift to a more immature basal signature, which is associated with enhanced metastatic potential and aggressiveness (Cheung et al., 2013). Consistent with a more basal-like phenotype, the authors observed the expansion of a subpopulation of cells expressing the basal marker CK14 in autophagy-deficient metastases.

The authors next assessed the impact of ablating autophagy on primary tumor growth. Consistent with previous studies implicating autophagy in promoting primary tumor progression, ablation of autophagy impaired the progression of primary tumors in contrast to its suppressive effect on the metastatic site. Primary autophagy-deficient tumors also exhibited expansion of CK14-positive basal-like cells with enhanced proliferation, similar to the metastatic site. However, the proliferation of CK14-negative cells was reduced in autophagy-deficient primary tumors, offering an explanation for their impaired growth.

What is the mechanism underlying the suppression of metastasis by autophagy? Previous studies have shown that shutdown of autophagy leads to the cellular accumulation of autophagy cargo receptors (ACRs), which modulate cell fate by acting as signaling scaffolds (Hernandez et al., 2014). Marsh et al. (2020) identified that the ACRs NBR1 and P62 accumulate in autophagy-deficient cells, which prompted them to assess their impact on metastatic outgrowth. Employing loss- and gain-of-function experiments, the authors found that NBR1 promotes metastatic progression. Notably, depletion of NBR1 completely abrogated the impact of ablating autophagy on metastatic outgrowth and expansion of CD14-positive basal-like cells, suggesting that autophagy suppresses metastasis by preventing the accumulation of NBR1.

To assess the human relevance of these findings, the researchers next investigated the correlation between an autophagy gene signature and the expression of genes associated with a stem cell/basal-like phenotype in breast cancer patients. Leveraging data from The Cancer Genome Atlas (Cancer Genome Atlas Network, 2012), they uncovered a negative correlation between the autophagy and basal signatures, consistent with autophagy suppressing the emergence of a stem-cell-like/basal phenotype. The authors also demonstrated that patients with high expression of an autophagy signature experience enhanced survival, consistent with autophagy suppressing metastasis.

Marsh et al. (2020) next assessed the effects of pharmacological modulation of autophagy. Interestingly, systemic treatment of metastasis-bearing mice with the autophagy inhibitor chloroquine did not impact metastatic progression. In contrast, administration of rapamycin, an inhibitor of the mammalian target of rapamycin complex 1 (mTORC1), which promotes autophagy, significantly suppressed the progression of metastasis and the expansion of CK14+ cells. To more specifically activate autophagy in metastatic breast cancer cells, the authors also genetically activated autophagy. Similar to the pharmacologic activation of autophagy, genetically enforcing autophagy suppressed metastatic progression. These data are highly significant in revealing promise for activation of autophagy as a therapeutic approach for suppressing breast cancer metastasis.

The elegant and comprehensive work by Marsh et al. (2020) provides compelling evidence supporting the context-dependent impact of autophagy in cancer—impacting arguably its most clinically meaningful stage: metastatic progression. Clinicians, trialists, and those in industry must strongly take the metastasis-suppressive role of autophagy uncovered by the authors into account when therapeutically targeting autophagy in cancer patients.

These remarkable findings unveil a number of key questions for future research: Does NBR1-signaling play a role not only in suppressing metastasis, but also in suppressing the reactivation of dormant breast cancer cells, which is the main cause of late breast cancer relapse (La Belle Flynn et al., 2019)? Does the metastasis-suppressing role of autophagy extend to other cancer types? It is interesting to note that ablation of autophagy induced the emergence of a basal phenotype in only a subpopulation of autophagy-deficient cells. This raises the question of what determines the threshold for such phenotype conversion, and which signals downstream of NBR1 mediate it. Lastly, a growing body of evidence points toward a link between cancer autophagy and anti-tumor immunity (Zhong et al., 2016). It will be important to elucidate whether the immune response could at least in part explain the metastasis-suppressive effect of autophagy on metastasis observed by the authors, given previous evidence that autophagy enhances anti-tumor immunity (Ladoire et al., 2016; Martins et al., 2012).

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Hippo Signaling: Autophagy Waits in the Wings

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Crosstalk between signaling networks can help coordinate diverse cellular functions. In this issue of *Developmental Cell*, Tyra et al. identify connections between the cell-growth-promoting transcription factor YAP/Yorkie and the autophagy-regulating kinase Ulk1/Atg1.

Growth and proliferation of cells require a steady diet of macromolecules and are rapidly curtailed in response to nutrient deprivation and other metabolic stresses. At the same time, these signals elicit an upregulation of autophagy, providing the cell with a temporary internal supply of nutrients and allowing for a restructuring of metabolic pathways. The mechanisms that coordinate these opposing growth and degradative activities remain incompletely understood, and likely have a major impact on cell physiology and survival. Connections between the signals that control autophagy and cell growth are beginning to emerge, and in this issue of *Developmental Cell*, Tyra et al. (2020) provide further insights into how these processes are interconnected.

The Hippo pathway was originally identified in *Drosophila* through the striking tissue overgrowth phenotypes resulting from mutations in Hippo or its downstream factors Salvador, Mats, and Warts (mammalian Sav1, Mob, and Lats1/2, respectively). Work in fly and mammalian cells ultimately identified the transcriptional co-activator Yorkie (Yki; YAP/TAZ in mammals) as the nuclear effector of this pathway (Zheng and Pan, 2019). In response to a variety of upstream signals, active Hippo signaling results in phosphorylation of Yki by Warts, leading to its nuclear exclusion and destabilization. Under favorable growth conditions, Yki interacts with the TEAD family of DNA binding factors to promote transcription of genes that drive cell growth and survival. Hyperactive YAP/TAZ activity has been implicated in numerous forms of cancer and other diseases.

Regulation of autophagy in response to nutrient availability is governed by an equally complex signaling network. The Ser/Thr kinase Atg1 (Ulk1/2 in mammals) plays a central role in the initial steps of autophagy induction, responding to changes in nutrient and energy levels via regulation by the mTOR and AMPK kinases and driving the formation of autophagosomes by phosphorylating a number of core autophagy regulators (Zachari and Ganley, 2017). Positive and negative feedback between components of this network combine to maintain autophagic activity within tightly prescribed limits.

In their new report, Tyra et al. (2020) first describe a series of genetic interactions between Yki and Atg1. Expression of mutant versions of Yki that are resistant to inactivation by Hippo signaling leads to overgrowth and disorganization of adult fly structures such as eyes and wings and in severe cases can result in lethality. Each of these phenotypes was found to be suppressed by co-expression of Atg1 or its upstream activator Acinus. Upregulation of the Yki-responsive genes *bantam, Ex,* and *Diap1* was also blocked by Atg1 co-expression. Depletion of Atg1 had the converse effect, suppressing lethality caused by knockdown of Yki. Importantly, Atg1 and Acinus also affected signaling at endogenous levels of Yki, as their targeted depletion in otherwise wild-type flies caused an increase in wing size and expression of Yki target genes, and their overexpression inhibited growth. Interestingly, manipulating expression of other autophagy-related genes did not consistently affect growth or patterning of fly tissues, suggesting that inhibition of Yki is an autophagy-independent function of Atg1.