ACKNOWLEDGMENTS

A.A. is the recipient of an Advanced Grant of the European Research Council and is supported by grants from the European Union (PRIORITY, NEURINOX), the Swiss National Foundation, and the Novartis Research Foundation. A.D.G. is supported by NIH Director's New Innovator Award DP2OD004417, and NIH grants R01NS065317 and R01NS073660. We thank Dr. Lili Guo for assistance with artwork.

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Self-Eating Limits EGFR-Dependent Tumor Growth

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http://dx.doi.org/10.1016/j.cell.2013.08.040

Autophagy is a cell-autonomous, catabolic process that plays context-dependent roles in tumor growth and progression. Wei et al. report that EGFR signaling promotes tumor growth through phosphorylation and functional inactivation of Beclin 1 and the consequent suppression of autophagy.

Macroautophagy (hereafter termed autophagy) is a degradative process that involves the encircling of cytoplasmic elements by a specialized endomembrane structure (the autophagosome), which delivers the cargo to lysosomes for degradation and recycling into cellular metabolic pathways. Autophagy supports cell survival during metabolic stress and maintains normal homeostasis by ridding the cell of protein aggregates and dysfunctional organelles (Choi et al., 2013). Although autophagy is clearly relevant to cancer biology, studies to date paint a rather muddled picture, which indicates that autophagy either suppresses or promotes tumor growth, depending on cancer subtype and the stage of tumor development (White, 2012). The report by Wei et al. (2013) in this issue of *Cell* defines a novel pathway through which the epidermal growth factor receptor (EGFR) suppresses autophagic activity to promote the growth of non-small-cell lung cancers (NSCLCs).

The core components of the autophagic machinery are conserved in eukaryotic cells. Two critical proteins involved in the initiation of autophagy are the class III phosphoinositide 3-kinase, VPS34, and the protein serine-threonine kinase, ULK1 (see Figure 1). VPS34 activation leads to the localized accumulation of phosphatidylinositol 3-phosphate, which stimulates endomembrane events required for autophagosome formation (Lorin et al., 2013). VPS34 activity is regulated, in part, through binding to Beclin 1, a scaffolding protein that has been identified as a haploinsufficient tumor suppressor (Choi et al., 2013; Lorin et al., 2013). Beclin 1 governs autophagy through interactions with proteins that either stimulate (ATG14L, UVRAG, and AMBRA1) or suppress (Bcl-2, Bcl-xL, and Rubicon) VPS34 activity. The scaffolding functions of Beclin 1 are directly modulated by a growing number of protein kinases, including mTOR, AMPK, ULK1, and AKT (Lorin et al., 2013; Russell et al., 2013).

The EGFR tyrosine kinase is overexpressed or mutationally activated in a broad range of human cancers, including NSCLC. Previous reports indicated that EGFR activation suppresses autophagy and that TKI exposure triggers increased autophagic flux in NSCLC cells (Fung et al., 2012; Han et al., 2011). However, the interplay between EGFR signaling and the autophagy machinery, as well as the role of autophagy in EGFR-driven tumor growth, remained unclear. Wei et al. (2013) demonstrate that Beclin 1

binds to and is a substrate for the activated EGFR tyrosine kinase. Tyrosine phosphorylation of Beclin 1 provokes disassembly of the Beclin 1-VPS34 complex and the association of Beclin 1 with two suppressors of autophagy, Rubicon and Bcl-2. The net result of this rearrangement of the Beclin 1 "interactome" (as termed by the authors) is that VPS34 activity and, in turn, autophagy, are suppressed in response to EGFR activation. Exposure of NSCLC cells to EGFR tyrosine kinase inhibitors (TKIs) restores the interaction of Beclin 1 with VPS34 and triggers an increase in autophagy. Notably, this mechanism of autophagy regulation is independent of EGFR-dependent mTOR complex 1 (mTORC1) activation, a well-established mechanism of autophagy downregulation. In the NSCLC setting, modulation of the Beclin 1 interactome appeared to be a dominant mechanism for autophagy suppression by the activated EGFR. This outcome may reflect a relatively inefficient coupling of EGFR signaling to PI3K signaling, possibly due to suboptimal expression of the HER3 subunit in the NSCLC cells (Sithanandam and Anderson, 2008). Interestingly, Wei et al. (2013) reported that Beclin 1 tyrosine phosphorylation is not induced by the PDGF receptor, which strongly activates the PI3K-mTOR pathway. Thus, the relative contributions of the Beclin 1-VPS34 versus PI3K-mTOR mechanisms to the regulation of autophagy likely vary among different receptor tyrosine kinase subtypes.

Wei et al. (2013) identified three conserved tyrosine residues in Beclin 1 that were phosphorylated by the EGFR tyrosine kinase. The authors posited that tyrosine phosphorylation at these sites promotes Beclin 1 homodimerization, dissociation of VPS34, and assembly of the inactive Beclin 1-Rubicon-Bcl-2 complex (Figure 1). Replacement of the targeted tyrosines in Beclin 1 with phosphomimetic glutamic acid residues generated a Beclin 1-EEE mutant that acted as a dominant-negative-inhibitor of VPS34 activity and autophagy. Importantly, Beclin 1-EEE-expressing NSCLC cells failed to increase autophagy during TKI exposure, which supports the hypothesis that this TKI response hinges on the dephosphorylation of Beclin 1.

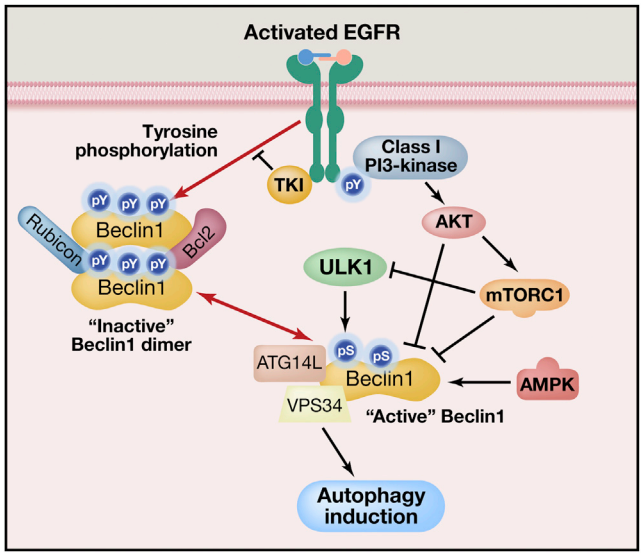

Figure 1. Multiple Protein Kinases Target Beclin 1 and Regulate the Beclin 1 Interactome

EGFR signaling activates the class I PI3K-AKT-mTORC1 pathway. AKT and mTORC1 phosphorylate Beclin 1 on serine residues, leading downregulation of VPS34 activity and autophagy. In contrast, the ULK1 and AMPK kinases stimulate autophagy by phosphorylating Beclin 1 and promoting the formation of the active Beclin 1-VPS34 complex. mTORC1 also suppresses autophagy initiation by phosphorylating and inhibiting ULK1. Wei et al. (2013) demonstrate (red arrows) that the EGFR tyrosine kinase phosphorylates Beclin 1 and drives the formation of Beclin 1 homodimers that do not support VPS34 activity, thereby inhibiting autophagy. These EGFR-dependent events are blocked by TKIs.

Tumor xenograft studies with NSCLC cells engineered to express mutated Beclin 1 proteins yielded striking results (Wei et al., 2013). Beclin 1-EEE-expressing NSCLC cells formed faster-growing tumors than those generated by wild-type cells or cells bearing the corresponding alanine substitution mutant of Beclin 1 (Beclin 1-AAA), which cannot be phosphorylated by the activated EGFR. Interestingly, Beclin 1-EEE tumors also exhibited higher levels of cell death than wild-type or Beclin 1-AAA tumors. This finding is consistent with the well-established role of autophagy in metabolic stress resistance; however, the proliferative advantage conferred by autophagy suppression apparently more than compensates for the increased rate of cell death in these Beclin 1-EEE tumors.

Histologic analyses of the Beclin 1-EEE tumors also yielded some unanticipated findings. Wei et al. (2013) observed that, whereas the wild-type and Beclin-AAA-expressing tumor tissues displayed the expected glandular architecture associated with lung adenocarcinomas, the Beclin 1-EEE xenografts exhibited a less well-differentiated phenotype that resembled adenosquamous lung carcinoma, a NSCLC subtype that carries a particularly poor prognosis (Filosso et al., 2011). Established Beclin 1-EEE tumors also displayed significant resistance to TKI treatment, suggesting that the impaired autophagic response to EGFR inhibition was causally related to drug resistance in these tumors. As Wei et al. (2013) acknowledge, it cannot be ruled out that Beclin 1-EEE interferes with other functions of endogenous Beclin 1 that contribute to tumor growth and TKI resistance in these studies.

The report by Wei et al. (2013) underscores the precept that the impact of autophagy on tumor growth and progression is highly context dependent (White, 2012). Furthermore, the study offers a cautionary note regarding the clinical application of autophagy inhibitors in patients receiving TKI therapy for NSCLC and other EGFR-linked cancers. A more complete delineation of the receptor tyrosine kinases that govern autophagy through tyrosine phosphorylation of Beclin 1 is clearly needed, as is an understanding of the parameters that dictate whether autophagy supports or limits the growth of different tumor types. Finally, emerging evidence that autophagy modulates tumor histology (Guo et al., 2013; Wei et al., 2013) adds yet another variable that demands further research if autophagy modulators are to be used safely and effectively in cancer.

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Intrinsic Activity of Odorant Receptors Guides Sensory Map Formation

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http://dx.doi.org/10.1016/j.cell.2013.08.037

Olfactory sensory neurons innervate the olfactory bulb in stereotyped patterns according to the odorant receptors they express. A study by Nakashima et al. in this issue demonstrates that the odorant receptor's level of intrinsic activity—in the absence of activating odorant—influences the guidance of olfactory axons to their targets.

Olfactory sensory neurons (OSNs) typically express just one odorant receptor (OR) from a repertoire of more than 1,000 OR genes (DeMaria and Ngai, 2010). OSNs expressing the same OR are distributed across large areas of the sensory epithelium and project their axons to common and spatially invariant sites—called glomeruli—in the olfactory bulb. Individual glomeruli receive innervation only from OSNs expressing the same OR; the spatial representation of ORs in the bulb forms the anatomical basis of the olfactory sensory map and reflects a remarkable feat of pattern formation during development. Targeting of olfactory axons along the olfactory bulb's dorsal-ventral and medial-lateral axes occurs through OR-independent mechanisms (DeMaria and Ngai, 2010). In contrast, "receptor swaps" in which the coding region of one OR gene is replaced with the coding region of another OR cause shifts in glomerular position along the anterior-posterior (AP) axis of the bulb (Wang et al., 1998). A tantalizing hypothesis emerging from these receptor swap experiments posits that the OR not only receives sensory information from small volatile molecules in the environment but also from axon guidance cues that determine where in the bulb the OSN's axon projects. This model, as appealing as it may seem, has yet to receive compelling experimental support.

If the OR does not function as an axon guidance receptor per se, perhaps it sets the responsiveness or "gain" of the OSN to classical axon guidance cues through its level of activity. But how? ORs are unlikely to encounter their cognate odorants in utero. Like other G-protein-coupled receptors, in the absence of ligand, ORs are intrinsically active, existing in equilibrium between an active and inactive state (Rosenbaum et al., 2009). Given the sequence diversity of ORs, it is not hard to imagine a similar diversity in the level of OR intrinsic activity based on each receptor's unique physical properties. Initial support for this model came from a demonstration that perturbations in cyclic AMP (cAMP) signaling—the second messenger pathway employed in olfactory sensory transduction—altered the projection of OSN axons along the AP axis of the olfactory bulb (Imai et al., 2006); decreased cAMP signaling led to aberrant projections toward the anterior bulb, whereas increased cAMP signaling led to projections posterior to the location of the normal glomerulus. Imai et al. (2006) further demonstrated that expression of Neuropilin1, a receptor for the repulsive axon guidance cue Semaphorin 3A, is regulated by cAMP (via protein kinase A) in developing OSNs, neatly tying together the OR and axon guidance. Direct evidence that activity of unliganded receptor influences OSN axon guidance—and does so in an OR-specific way—was nonetheless lacking.