AUTOPHAGY
2017, VOL. 13, NO. 7, 1241–1243
https://doi.org/10.1080/15548627.2017.1310361

Taylor & Francis
Taylor & Francis Group

AUTOPHAGIC PUNCTUM

Microenvironment and tumors—a nurturing relationship

Nadja Sandra Katheder[a,b] and Tor Erik Rusten[a,b]

[a]Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Montebello, Oslo, Norway; [b]Centre for Cancer Biomedicine, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Norway

ABSTRACT

When exposed to adverse environmental conditions, cells degrade their own content to recycle cellular building blocks through a process called autophagy. A large body of literature has connected autophagy to cancer, but most studies up until now focused on its function in transformed cells. In her thesis, Nadja Katheder dissected the role of autophagy in a well-characterized neoplastic in vivo tumor model in Drosophila and demonstrates a novel non-cell-autonomous requirement of this process for tumor growth. Neighboring epithelial cells and distal tissues increase autophagy in the presence of a malignant tumor. Pharmacological autophagy inhibition reduces tumor growth and genetic ablation of autophagy in the microenvironment reveals a tumor-supportive role of this process in this specific cell population. Tumor cells are metabolically stressed and induce autophagy in their neighbors through a TNFα-JNK-IL-6 signaling cascade. Moreover, they are dependent on amino acid import to sustain their proliferation, which indicates a coupling of metabolism between these two cell populations. Finally, allografted growth-impaired tumors from autophagy-deficient donor animals resume growth in an autophagy-competent host. Together, the results described in this thesis highlight the tumor-promoting role of autophagy the microenvironment and show that cancer cells engage their epithelial neighbors as essential contributors aiding their own growth.

ARTICLE HISTORY
Received 13 March 2017
Revised 17 March 2017
Accepted 17 March 2017

KEYWORDS
amino acid transporter; autophagy; *Drosophila*; hippo; interleukin-6; JNK; microenvironment; ras; scribble

The role of macroautophagy/autophagy in cancer has been the subject of a myriad of studies attempting to dissect the complex and often contradictory functions of this process at different stages of tumorigenesis. Generally, autophagy is considered to have a tumor-suppressive role in healthy cells by maintaining cellular homeostasis and preventing transformation, whereas it adopts a tumor-promoting role in established tumors. However, efforts have so far predominantly focused on the cell-autonomous contribution of autophagy to tumor development and growth.

We showed recently that autophagy also adopts an important non-cell-autonomous function in the microenvironment to support tumor growth in a *Drosophila* model of malignant ras^{G12V}-driven cancer. Loss of the tumor suppressor *scrib* (*scribbled*), encoding a protein required for epithelial cell polarity, cooperates with Ras^{G12V}, resulting in aggressive and invasive neoplastic tumors. We observed that Cherry-Atg8a structures are massively enriched in cells adjacent to transformed ras^{G12V} $scrib^{-/-}$ cells in the eye-imaginal disk epithelium of larva (Fig. 1). Furthermore, distal organs such as the gut, adipose tissue and muscles also upregulate autophagy, suggesting a systemic response to the presence of malignant growths. To address the role of this non-cell-autonomous autophagy (NAA) in the development of ras^{G12V} $scrib^{-/-}$ tumors we first treated larvae with chloroquine, which reduces tumor size and invasion efficiently. Due to the systemic and potentially autophagy-independent effects of this drug we then used genetic clonal analysis techniques to specifically block autophagy in different cell populations to dissect the function of NAA more precisely. Removal of *atg13* within the tumor cells diminishes tumor size only marginally, whereas ablation in cells adjacent to ras^{G12V} $scrib^{-/-}$ clones significantly hampers growth. Interestingly, autophagy-deficient neighbors also reduce invasiveness of transformed cells. Tumor growth and invasiveness is reduced even further in *atg13*-mutant animals, indicative of a systemic contribution. Flow cytometry experiments and caspase antibody staining showed that diminishes tumor burden is a consequence of reduced proliferation, not increased apoptosis. Rescue experiments with a genomic construct covering the endogenous *atg13* locus confirmed the specificity of the phenotypes. Unexpectedly, *atg13*-deficient tumors facing $atg14^{-/-}$ neighbors are also attenuated and tumor volume is reduced to a similar extent as seen in Atg13-deficient animals. We also performed an eye-specific rescue of autophagy function, which results in a partial restoration of tumor growth, thereby further substantiating that local autophagy is crucial for early tumor development but also hinting at a potential systemic input to tumor formation at later stages.

CONTACT Tor Erik Rusten torr@rr-research.no Institute for Cancer Research, The Norwegian Radium Hospital, Ullernschaussen 70, 0379, Oslo, Norway.
Punctum to: Katheder NS, Khezri R, O'Farrell F, Schultz SW, Jain A, Rahman MM, Schink KO, Theodossiou TA, Johansen T, Juhász G, et al. Microenvironmental autophagy promotes tumor growth. Nature. 2017 Jan 19; 541(7637):417-420

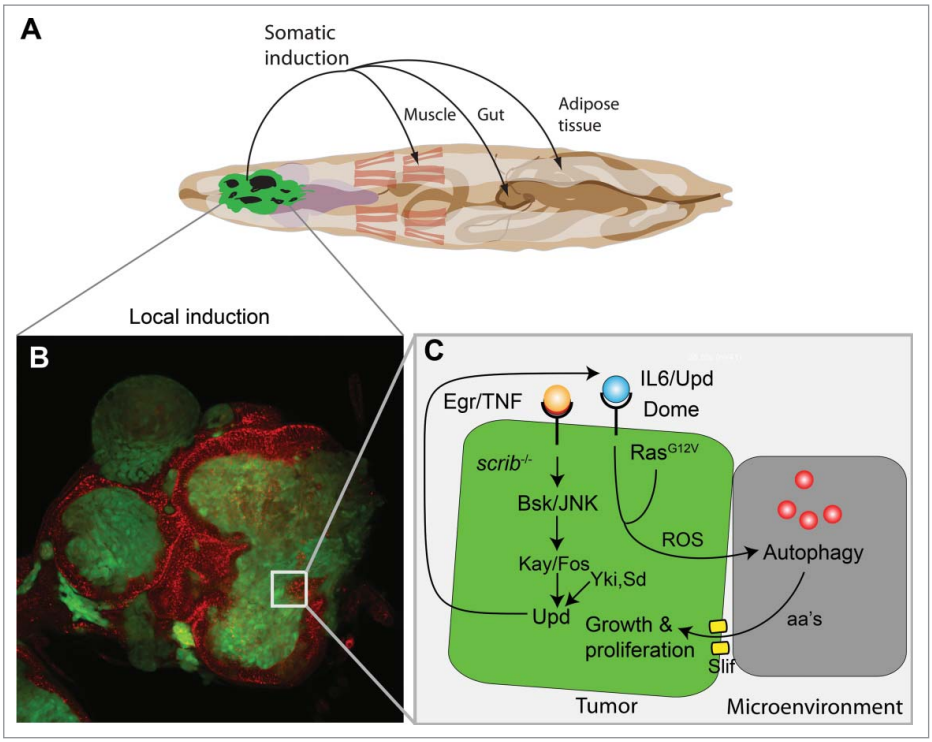

Figure 1. Tumor-microenvironment interaction and growth support by autophagy. (A) Cartoon depicting somatic and local induction of autophagy in response to malignant *ras*^{G12V}, *scrib* tumor cells (green) generated in the eye. (B) Confocal image of eye imaginal disk epithelium with *transformed cells* (green, GFP), inducing a striking autophagy stress response in epithelial cells of the microenvironment (red, Cherry-Atg8a-positive autophagic structures). (C) Model depicting transformed cells with active Ras^{G12V}- Bsk/JNK-, Hpo/hippo (Yki,Sd)-, IL6- and TNF signaling loop resulting in metabolic stress, ROS production and non-autonomous induction of autophagy in the microenvironment. Autophagy in the microenvironment in turn promotes tumor cell proliferation, likely by supplying amino acid building blocks through amino acid transporters. ROS, reactive oxygen species.

Several elegant studies have dissected signaling in *ras*^{G12V} *scrib*$^{-/-}$ clones and identified Bsk/JNK and impaired Hippo signaling as the main drivers of tumorous growth. Epistasis experiments showed that NAA depends on tumor-intrinsic Bsk/JNK signaling and transcriptional activity through Kay/Fos, as well as impaired Hpo (hippo) signaling and subsequent activity of the transcription factor Sd (scalloped) and its co-activator Yki (yorkie). Previous studies have shown that these signaling pathways converge and drive the expression of IL6 cytokines, also known as Upd1 (unpaired 1), Upd2 and Upd3, which serve as ligands for the Dome (domeless) receptor Hop/JAK-Stat92e/STAT signaling cascade. Co-expression of Ras^{G12V} with Upd1 and Upd3 is sufficient to trigger NAA, but suppression of NAA by blocking tumor-intrinsic Hop/JAK-Stat92e/STAT signaling showed that these cytokines act in an autocrine fashion on the tumor tissue itself, rather than on neighboring cells. These findings were further corroborated by removal of Stat92e/STAT in cells surrounding *ras*^{G12V} *scrib*$^{-/-}$ clones, which fails to abrogate NAA. Importantly, reduced tumor size is not the cause of NAA absence, as clones where growth-promoting class I PI3K signaling is interrupted still induce a robust autophagic response in the neighboring tissue. However, induction of systemic autophagy seems to be regulated differently from local NAA, as blocking Bsk/JNK-signaling in the eye disk does not suppress distal responses.

A combination of correlative light and electron microscopy, flow cytometry and confocal microscopy uncovered a large amount of damaged mitochondria and elevated ROS production in tumor cells, indicative of metabolic stress in this cell population. Earlier reports have identified ROS as a potent inducer of autophagy, which led us to investigate the potential role for ROS as a trigger of NAA. Indeed, genetic production of ROS by knockdown of the electron transport chain complex I subunit ND-75 is sufficient to provoke NAA in the wing disk, rendering it a likely candidate molecule for NAA induction. However, attempts to scavenge ROS genetically or pharmacologically fail to suppress NAA, although it should be noted that we were unable to verify the efficiency of these tools.

Measurements of metabolic oxidative phosphorylation capacity by Seahorse analysis indicated maximal respiration of *ras*^{G12V} *scrib*$^{-/-}$ cells and prompted us to assess uptake of glucose, which was strongly upregulated. Since autophagy is well known for its function in breaking down proteins and recycling amino acids (aa), we tested whether aa uptake also contributed to tumor growth. Knockdown of the cationic aa transporter Slif (slimfast) reduces tumor size dramatically, highlighting the dependency of *ras*^{G12V} *scrib*$^{-/-}$ tumors on import of externally provided aa and hypothetically linking up tumor cell metabolism with autophagy in neighboring cells. In support of our findings a recent study showed autophagy induction in pancreatic stellate cells, a cell type found abundantly in pancreatic cancer stroma, and identified alanine as a secreted product taken up by cancer cells, showing that metabolism of these 2 cell populations indeed seems to be coupled.

Lastly, we performed allograft experiments to test whether development of tumors is dependent on cues of the local

environment. Allograft size is reduced by chloroquine treatment and in atg14-hypomorph hosts, suggesting that host autophagy can also support growth of tumor tissue. Interestingly, tumor tissue from *atg13*-deficient animals resumes proliferation in wild-type hosts, showing that growth attenuation is reversible. Whether allografted tumors create their own new ectopic microenvironment or whether distant tissues also contribute to tumor growth remains to be established.

Taken together, our results clearly demonstrate that NAA plays an important role in supporting early tumor growth in vivo. These findings underscore the importance of taking tumor-microenvironment interactions into consideration when addressing the role of autophagy in cancer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.