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Blocking tumor growth by targeting autophagy and SQSTM1 in vivo

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Autophagy is a highly conserved cellular process for degradation of bulk cytoplasmic materials in response to starvation and maintenance of cellular homeostasis. Dysfunction of autophagy is implicated in a variety of diseases including cancer. In a recent study, we devised a system for inducible deletion of an essential autophagy gene *Rb1cc1/Fip200* in established tumor cells in vivo and showed that *Rb1cc1* is required for maintaining tumor growth. We further investigated the role of the accumulated SQSTM1 in *Rb1cc1*-null autophagy-deficient tumor cells. To our surprise, the increased SQSTM1 was not responsible for the inhibition of tumor growth, but rather supported the residual growth of tumors (i.e., partially compensated for the defective growth caused by *Rb1cc1* deletion). Further analysis indicated that SQSTM1 promoted tumor growth in autophagy-deficient cells at least partially through its activation of the NFKB signaling pathway. A working model is proposed to account for our findings, which suggest that targeting both autophagy and the consequently increased SQSTM1 may be exploited for developing more effective cancer therapies.

Keywords: autophagy, mouse models, SQSTM1, targeted cancer therapy, tumor growth

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Autophagy is a highly conserved process in which bulk cytoplasmic materials are sequestered and delivered to lysosomes for degradation. Besides its recycling function in nutrient or energy starvation, autophagy is increasingly recognized as a quality control mechanism for cellular homeostasis. Under various stress conditions, autophagy is activated to clear protein aggregates, impaired organelles, and intracellular pathogens. Dysfunctions in autophagy have been associated with different diseases including neurodegenerative diseases, inflammatory diseases, and cancers. Due to its impacts on a multitude of cellular functions, autophagy has been shown to play both tumor suppressive and promoting functions under different contexts and in different models.

RB1CC1/FIP200 encodes a highly conserved protein, which is a component of the ULK1-ATG13-RB1CC1/FIP200 complex essential for the induction of mammalian autophagy. In a previous study, we showed that conditional knockout (cKO) of *Rb1cc1* decreased mammary tumor growth and metastasis driven by the PyMT oncoprotein, thus providing the first evidence for a pro-tumorigenic role for autophagy in animals with an intact immune system. Nevertheless, one limitation of the study is that autophagy is inactivated before tumor development (i.e., cKO of autophagy genes in the embryonic stage when a particular Cre is expressed). Therefore, this and several other recent studies using similar strategies to delete other autophagy genes such as *Atg5* or *Atg7* in lung and pancreatic cancers cannot precisely evaluate the role of autophagy in established tumors, which is particularly relevant information for targeting autophagy in cancer therapy (i.e., blocking autophagy after detection of tumors).

To overcome this limitation in previous studies and evaluate the role of autophagy in established tumors, we designed an inducible system to delete *Rb1cc1* after tumor development in vivo in the recent study under discussion. Using this system, we demonstrated that acute disruption of autophagy by *Rb1cc1* deletion in established tumors

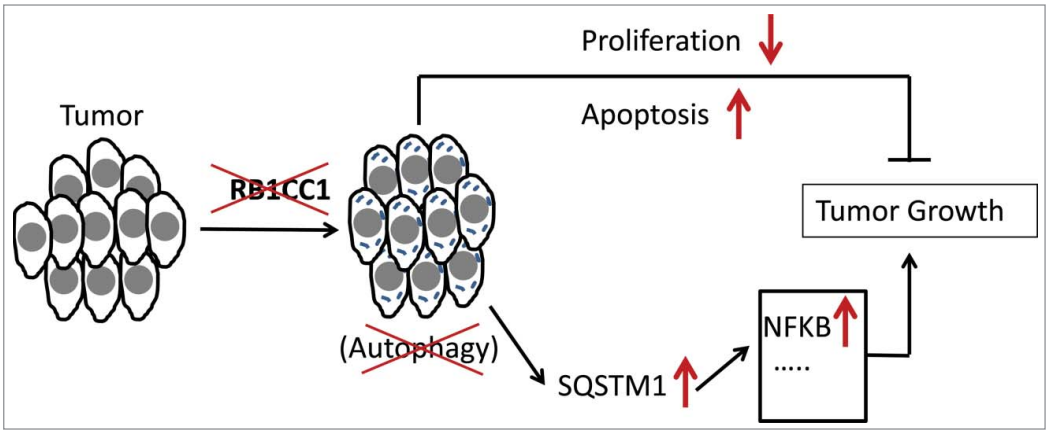

Figure 1. Autophagy deficiency caused by *Rb1cc1* deletion in established tumors causes decreased proliferation and increased apoptosis, which contribute to tumor inhibition. However, SQSTM1 accumulates after autophagy disruption. Increased SQSTM1 can stimulate tumor growth under the background of autophagy deficiency by upregulating NFKB and other pathways. Therefore, further inhibition of the SQSTM1 pathway once autophagy is disrupted will cause a synergistic effect on tumor inhibition.

from transformed MEFs as well as PyMT-driven mammary tumor cells dramatically blocks their growth in vivo. These results clearly show a positive role for autophagy in tumor growth and further validate the rationale to use autophagy inhibitors in the design of novel cancer therapies.

We then focused on investigating the mechanisms of the reduced tumor growth upon autophagy inhibition, especially the potential role of accumulation of SQSTM1 observed in *Rb1cc1* cKO tumor cells. SQSTM1 is an autophagy receptor molecule, but is also implicated in the regulation of various intracellular signaling pathways. SQSTM1 localizes to sites of autophagosome formation and serves as a receptor by binding to LC3 on the phagophore membranes and ubiquitinated cargo proteins. SQSTM1 itself is also an autophagy substrate, and therefore accumulates as protein aggregates in autophagy-deficient cells. However, the pathophysiological significance of this increased SQSTM1 expression in autophagy-deficient tumor cells was not clear. We therefore generated tumor cells with inducible *Sqstm1* knockdown as well as *Rb1cc1* cKO. To our surprise, we found that *Sqstm1* knockdown does not rescue the reduced tumor growth upon *Rb1cc1* deletion, but rather further decreases their growth in vivo. Conversely, we also showed that inducible re-expression of *Sqstm1* in *Sqstm1* KO cells enhances the growth of *Rb1cc1*-null, autophagy-deficient tumors, but has little effect on autophagy-competent tumors without *Rb1cc1* deletion. These results indicated that accumulated SQSTM1 in *Rb1cc1* cKO tumor cells is not responsible for their decreased tumor growth, but rather may contribute to the residual growth of autophagy-deficient tumors. These findings raise the interesting possibility of targeting both autophagy and SQSTM1 for potentially more effective cancer therapies.

Although SQSTM1 expression has been linked to tumor growth in previous studies, our recent results were the first to demonstrate its specific role in autophagy-deficient tumor cells. Therefore SQSTM1 accumulation can be viewed as (perhaps part of) tumor cell compensatory changes for autophagy inhibition. Besides its role as a receptor in autophagy, SQSTM1 has been shown to regulate a number of other intracellular signaling pathways. Our further analysis showed that SQSTM1 promotes tumor growth in autophagy-deficient cells at least partially through its activation of the NFKB signaling pathway. Future studies will be needed to determine whether other pathways affected by SQSTM1 may also play a role, and if so, how the various pathways collaborate in mediating the function of SQSTM1 in promoting tumor growth in autophagy-deficient cells. It will be particularly interesting to determine whether the KEAP1-NFE2L2/Nrf2 pathway is involved in mediating SQSTM1 action in our system, as this has been shown to be responsible for the liver phenotypes caused by SQSTM1 accumulation in autophagy-deficient cells.

Overall, as shown in a working model (**Fig. 1**), our recent studies provide the evidence that autophagy is required for established tumor growth, and increased SQSTM1 contributes to the growth of autophagy-deficient tumors at least partially through its regulation of the NFKB pathway. Therefore, targeting both autophagy and the consequently elevated SQSTM1 may be exploited to develop potentially more effective cancer therapies.