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Pro-tumorigenic function of autophagy in mammary oncogenesis

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Autophagy is a highly conserved catabolic cellular process by which cells degrade intracellular constituents in lysosomes, and its dysfunctions have been associated with a variety of human diseases including cancer. Previous studies have linked autophagy to both tumor-suppressive and promoting functions in different contexts, although the pro-tumorigenic function of autophagy has not been examined directly in breast or other cancers in animal models with intact immune functions in vivo. FIP200 (focal adhesion kinase family interacting protein of 200 kD) is a component of the ULK1-Atg13-FIP200-Atg101 complex that is essential for the induction of mammalian autophagy. In our recent study, we show that conditional knockout (KO) of *FIP200* in the well-characterized MMTV-PyMT mouse model of human breast cancer significantly suppresses mammary tumorigenesis and progression. Similar to a number of recent studies in Ras-transformed cells, our studies revealed the importance of autophagy in promoting tumorigenesis through regulation of tumor cell glycolysis and proliferation. In addition to the intrinsic defects in proliferation of FIP200-null tumor cells, we also showed that *FIP200* deletion in mammary tumor cells triggers increased host antitumor immune surveillance, which also contributes to the decreased mammary tumorigenesis and progression. Our study provides the first direct demonstration of a pro-tumorigenic role of autophagy in oncogene-driven tumor models with intact immune functions in vivo. The models also suggest FIP200 and other autophagy proteins as potential therapeutic targets for cancer treatment, and raise a number of questions for future studies on the potentially dual functions of autophagy in promoting and suppressing tumorigenesis under different conditions in vivo.

Keywords: autophagy, FIP200, conditional knockout, mouse models, breast cancer

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Cancer development and progression is a complex biological process that is controlled by multiple basic cellular functions such as proliferation, migration, differentiation and apoptosis. In recent years, the potential role of autophagy in cancer has received increasing attention both as a basic biological question and because of possible applications in novel cancer therapies. Autophagy is a highly conserved catabolic cellular process by which cells degrade intracellular constituents in lysosomes. Unlike apoptosis, which is generally considered as a tumor- suppression mechanism through induction of cancer cell death, however, autophagy has a more complex role in cancer. Because of its basic function in maintaining cellular nutrient and energy homeostasis, disruption of autophagy causes increased accumulation of ubiquitin aggregates, p62/SQSTM1, which targets ubiquitinated protein aggregates to autophagosomes, and damaged mitochondria and reactive oxygen species (ROS), as well as an increase in DNA damage, and cell death. In apoptosis-defective cells, however, the increased DNA damage as a result of autophagy inhibition could lead to neoplastic transformation. These results suggesting a tumor- suppressor function of autophagy through limiting oxidative stress and DNA damage are supported by the earlier observation of spontaneous development of multiple tumors in animals with

haploinsufficiency of *becn1*, which encodes an essential autophagy protein. On the other hand, several other studies show that pharmacological or genetic inhibition of autophagy can sensitize tumor cells to the cytotoxic effects of chemotherapy and ionizing irradiation to benefit cancer treatments. Recent studies suggest that autophagy may promote cancer cell survival and proliferation through its role in aerobic glycolysis and the tricarboxylic acid cycle in oncogenic Ras-transformed cells. However, despite suggestive data from studies using cancer cell lines and xenograft models in nude mice, a pro-tumorigenic role of autophagy has not been examined directly in breast or other cancers in animal models with intact immune functions in vivo.

FIP200 (FAK-family interacting protein of 200 kDa) encodes an evolutionarily conserved protein characterized by a large coiled-coil region containing a leucine zipper motif. It was initially discovered by its interaction with focal adhesion kinase (FAK) and its related kinase Pyk2, and later identified as a component of the ULK1-Atg13-FIP200-Atg101 complex that is essential for the induction of mammalian autophagy. Recent studies by us and others have shown a role of FIP200 in several other signaling pathways, and also demonstrated that *FIP200* deletion results in embryonic lethality, and conditional KO of *FIP200* in several other tissues leads to defects largely overlapping with those observed in mutant mice with deletion of other autophagy genes (e.g., *Atg5* and *Atg7*). In our recent study, we generated a conditional KO of *FIP200* in the well-characterized MMTV-PyMT mouse model of human breast cancer to investigate the potential pro-tumorigenic functions of autophagy in vivo. Similar to deletion of *FIP200* or other autophagy genes in other cells, inactivation of *FIP200* results in multiple autophagy defects including accumulation of ubiquitinated protein aggregates and p62, deficient LC3 conversion, and increased numbers of mitochondria with abnormal morphology in mammary tumor cells. More importantly, analysis of these mouse models showed that inhibition of autophagy by *FIP200* deletion significantly suppresses mammary tumorigenesis and progression, providing the first direct demonstration of a pro-tumorigenic role of autophagy in oncogene-induced tumorigenesis and progression in animals with an intact immune system.

Similar to a number of recent studies in Ras-transformed cells, our studies revealed the importance of autophagy in promoting tumorigenesis through regulation of tumor cell glycolysis and proliferation. Decreased proliferation was found in FIP200-null primary mammary tumors in vivo as well as isolated tumor cells in vitro. Moreover, expression of cyclin D1 is reduced in the FIP200-null tumor cells, and re-expression of ectopic cyclin D1 rescues the deficient proliferation of these cells. We also found an impaired glucose uptake and intracellular lactate production in FIP200-null tumor cells, suggesting a compromised glycolysis in these cells. Given the increased requirement of glycolysis for malignant cell proliferation (Warburg effect), these results suggest that autophagy deficiency may regulate proliferation through controlling glycolysis in tumor cells. Nevertheless, current data remain as strong correlations in our and the other recent studies, and this interesting possibility will still need to be verified in the future.

One interesting discrepancy in our studies is the observation of the increased tumor cell apoptosis in early primary tumors in vivo, but not in isolated tumor cells in vitro. Although previous studies in the nervous system show increased apoptosis upon neural-specific deletion of *FIP200* or other autophagy genes like *Atg5* and *Atg7*, the possible cell-autonomous effect was not analyzed directly using isolated neurons in vitro. Indeed, studies using primary MEFs show no difference in apoptosis upon *FIP200* deletion in basal conditions, even though FIP200-null MEFs exhibit increased sensitivity to a variety of agents that induce apoptosis. Therefore, it is possible that the increased apoptosis observed in vivo is due to unique microenvironmental stresses (e.g., hypoxia, nutrient deprivation, or increased immune surveillance, see below) not present in ex vivo tumor cell cultures.

Another major finding of our studies is that *FIP200* deletion in mammary tumor cells triggers increased host antitumor immune surveillance, which also contributes to the decreased mammary tumorigenesis and progression in addition to the intrinsic defects in proliferation of FIP200-null tumor cells. We found that a very prominent feature of the genome-wide alterations in FIP200-null tumor samples is increased expression of genes encoding proteins involved in response to interferon (IFN) stimulation and other aspects of the immune response. It is already known that upregulated IFN signaling plays an antitumor function. Therefore, our data suggested that *FIP200* deletion might trigger enhanced antitumor immune surveillance, which may also contribute to the suppression of mammary oncogenesis. We demonstrated that increased expression of many target genes of type I (IFNα/β) and type II (IFNγ) is caused by increased infiltration of immune cells, especially IFN-γ-producing $CD8^+$ T cells in FIP200-null tumors, rather than by increased sensitivity of KO tumor cells to IFN stimulation. Several recent studies show that autophagy deficiency could sensitize the cellular response via RIG-I-like receptor (RLR) signaling to induce type I IFN production. Similarly, we found that *FIP200* deletion causes a sensitized cell response, i.e., increased expression of IFN-β and chemokine CXCL10, upon stimulation by poly(I:C), a synthetic dsRNA. Thus, it is possible that increased expression of chemokines including CXCL10 in FIP200-null tumor cells triggered by foreign RNA (e.g., from dead cells in the tumor microenvironment) could be responsible for the increased infiltration of effector T cells. Moreover, the infiltrated effector T cells could further stimulate the secretion of chemokines including CXCL10 from mammary tumor cells, creating positive feedback amplification for the increased antitumor immune surveillance in FIP200 conditional KO mice in vivo.

In summary, our study demonstrated that suppression of autophagy by *FIP200* deletion inhibits mammary tumorigenesis and progression by both impairing tumor cell proliferation and inducing increased immune surveillance. These results provide strong support for a pro-tumorigenic role of autophagy in oncogene-driven

tumor models in vivo and also suggest FIP200 and other autophagy proteins as potential therapeutic targets for cancer treatment. However, many interesting questions still remain regarding the complex functions of autophagy in cancer. Further studies are needed to clarify the molecular and cellular mechanisms by which autophagy regulates cancer in a positive vs. negative manner. For example, recent studies by others show that inactivation of *Atg5* or *Atg7* leads to spontaneous development of benign liver tumors. Although their deletion in mammary epithelium or several other tissues does not lead to spontaneous tumor formation, will *FIP200* KO in hepatocytes also lead to liver tumor formation? If so, will these be benign tumors, as in the case of *Atg5* and *Atg7* KO, or will these progress to malignant hepatocarcinomas as in *becn1* inactivation? Likewise, is the increased immune surveillance upon *FIP200* deletion due to defective autophagy, or potentially unique functions of FIP200 through its interaction with other signaling pathways? Future studies of multiple key autophagy genes in appropriate mouse models of human breast and other cancers will help to clarify these questions as well as potentially other functions of these genes, which may open up new avenues of studies on the crosstalk of autophagy with other cellular functions and signaling pathways.