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The roles of BECN1 and autophagy in cancer are context dependent

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Malignant tissue contains a rare population of multi-potent cells known as cancer stem-like cells (CSCs). Autophagy is an important mechanism in cancer cell survival and tumor growth; it can both suppress malignant transformation and promote the growth of established cancers. However, the molecular mechanisms underlying the tumor-promoting and tumor-suppressing functions of autophagy in CSCs are not understood. Our work demonstrates that a prosurvival autophagic pathway is critical for breast CSC maintenance. Notably, we provide new evidence for the existence of two separate, context-dependent, autophagic programs that are regulated in opposite ways by BECN1.

Keywords: breast cancer, autophagy, cancer stem-like/progenitor cell, Beclin 1

Abbreviations: BCSCs, breast cancer stem-like/progenitor cells; ALDH1, aldehyde dehydrogenase 1

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The double-edged functions of autophagy in cancer have raised new issues. The tumor mass is heterogeneous, so which subpopulation of cancer cells is addicted to autophagy? What mechanism is responsible for regulating the autophagy process in these various cells? It is important to know this, because blocking this process can be expected to kill autophagy-addicted cancer cells.

CSCs have been identified in many different types of solid tumor, including breast cancer. CSCs are not only the source of the tumorigenesis, but also may be responsible for tumor progression, metastasis, and resistance to therapy, as well as subsequent tumor recurrence. Breast cancer stem-like/progenitor cells (BCSCs) form spherical clusters (known as "mammospheres") in suspension cultures due to their self-renewal capacity; they carry the $CD44^+/CD24^-$ phenotype and display high aldehyde dehydrogenase 1 (ALDH1) activity ($ALDH1^+$). An appropriate CSC model is critical for analyzing autophagy and its involvement in CSC biology.

Our recently published results show that autophagic flux under both basal and starvation conditions is more robust in mammospheres derived from human primary cells and human breast cancer cell lines. It has been demonstrated that substratum detachment promotes autophagy as a survival mechanism. We analyzed the percentage of $ALDH1^+$ cells among the MCF-7 control and MCF-7 ATG7 knockdown cells before and after detaching the cells for 24 h. The data from these experiments show that there is no change in the percentage of $ALDH1^+$ after detachment in either cell population. To further confirm our results, we used an ALDEFLUOR assay to isolate the CSC/progenitor cell population derived from MCF-7 mammospheres. Western blot analysis shows that autophagic flux is markedly higher in $ALDH1^+$ cells. We also found that the lower survival in autophagy-deficient cells (MCF-7 ATG7 knockdown cells) during detachment does not contribute to an ultimate deficiency in mammosphere formation. These findings suggest that under both basal and starvation conditions, the CSC/progenitor phenotype displays higher autophagic flux than the non-CSC/progenitor phenotype.

The question is raised as to why CSC cells have elevated autophagy even under nutrient-replete conditions. Many recent studies have demonstrated that the elevated basal autophagy seen in tumors is critical for the cellular metabolism of cancers. Tumor cells require elevated energy production as well as increased

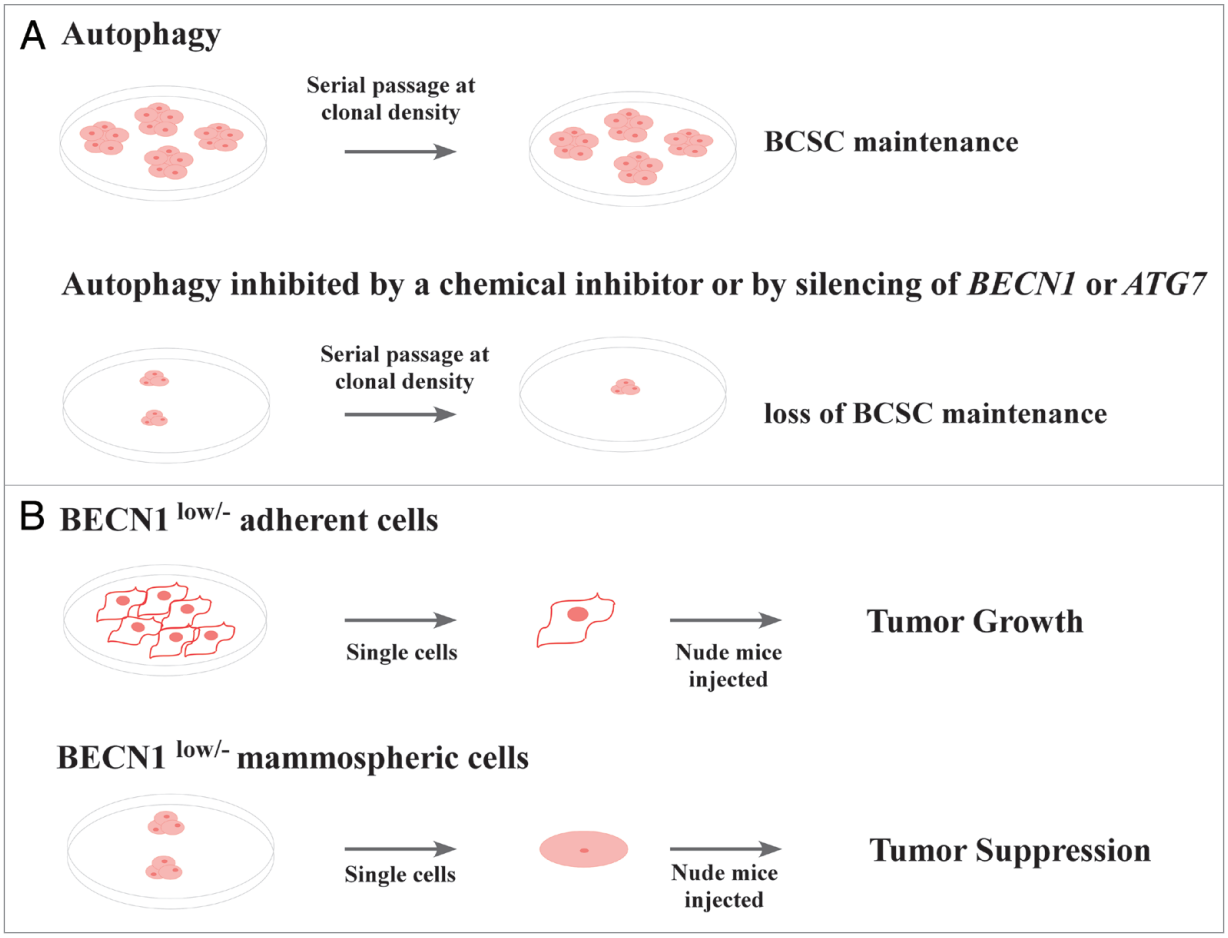

Figure 1. The prosurvival autophagic pathway is critical for maintaining cancer stem-like cells and the tumorigenicity of BCSCs. (**A**) A prosurvival autophagic pathway is critical for cancer stem-like cell maintenance. The number of mammospheres formed after serial passages at clonal density reflects the self-renewal capacity of primitive CSC, whereas the size of the mammospheres reflects progenitor cell proliferation. Autophagy inhibition using chemical inhibitors of autophagy and knockdown of the expression of essential autophagy genes, including *ATG7* and *BECN1*, results in a decrease in the size and number of mammospheres, suggesting that BECN1 is critical for maintaining the proliferation of BCSCs. (**B**) The autophagy protein BECN1 plays a dual role (tumor suppressor/tumor growth promoter) in tumor development.

protein, lipid, and nucleic acid synthesis to increase their biomass. Autophagy, at the most basic level, provides BCSCs with a large number of raw materials for multiple metabolic pathways, and through the degradation of proteins it can provide a pool of amino acids that can be used for anabolic reactions or energy production; this may be an important reason why BCSCs have a powerful proliferation and multidifferentiation potential as well as being able to produce distant metastasis. From these findings as a whole, we conclude that a high level of autophagy is a hallmark of BCSCs. However, which autophagy-supplied substrates are important for sustaining the metabolism of CSCs needs further investigation. This concept of altered tumor metabolism in CSCs is an area of active research, and efforts are under way to exploit this for therapeutic purposes.

The first indication that autophagy could play a role in tumor suppression came from looking at mice with allelic loss of the essential autophagy gene *Becn1*. These mice are partially defective for autophagy, and develop liver tumors with advancing age. These tumors arising from allelic loss of *Becn1* do not undergo loss of heterozygosity, suggesting that obligate haploinsufficiency occurs, and that the tumor cannot tolerate the loss of *Becn1* and autophagy.

Interestingly, we found that BECN1 (also known as Beclin 1) expression is upregulated in mammospheres. The number of mammospheres formed after serial passages at clonal density reflects the self-renewal of primitive CSCs, whereas the size of the mammospheres reflects progenitor cell proliferation. Knockdown of BECN1 in several breast carcinoma cell lines results in a decrease in the size and number of mammospheres formed, suggesting that BECN1 is critical for maintaining the proliferation of BCSCs.

The link between autophagy and the maintenance/proliferation of BCSCs was further confirmed using chemical inhibitors of autophagy and knocking down the expression of ATG7 (**Fig. 1A**). We also found that decreased survival in autophagy-deficient cells during detachment does not contribute to an ultimate deficiency in mammosphere formation. Accordingly, our data suggest that maintaining CSCs

by autophagy promotes the stem cell phenotype.

In addition, the incidence of tumor formation is lower in nude mice injected with several adherent control cells than in those injected with adherent BECN1 knockdown cells, which is consistent with findings that BECN1 depletion in monolayer cultures and mouse models increases tumorigenesis (**Fig. 1B**, top). Despite these early data implicating BECN1 in the suppression of breast cancer, our data using mammospheres and nude mice models support the idea that the suppression of autophagy in breast CSCs/progenitor cells by BECN1 depletion inhibits tumorigenesis (**Fig. 1B**, bottom). These findings taken together suggest that BECN1-mediated autophagy may be protumorigenic in BCSCs. Further investigations are required to investigate the signaling mechanism through which BECN1 plays a dual role (tumor suppressor/tumor growth promoter) in tumor development.

Currently, nearly 20 clinical trials exploring anti-autophagy strategies in a variety of human cancers are ongoing. The majority of these trials employ a combination of cytotoxic chemotherapies and targeted agents. However, the emerging complexity of autophagic pathways implies that the application of autophagy inhibitors may not be as effective as originally hoped, as both tumor-promoting and tumor-suppressing pathways can be affected in a context-dependent manner. Looking ahead, it will be imperative to understand the role of autophagy in different contexts and to develop autophagy inhibitors capable of targeting them selectively. This means that it will be increasingly important to identify which cancer cells and in which context are sensitive to anti-autophagy therapy. It is worth noting that a combination of autophagy inhibition with conventional and targeted agents may be synergistic, and thus sensitize BCSCs to the conventional therapy.