Editorial

Hitting the brakes on autophagy for overcoming acquired resistance in triple negative breast cancer

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Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, and it is defined by the lack of expression of estrogen (ER), progesterone (PR) and human epidermal growth factor (HER2) receptors. TNBC is characteristically aggressive with high recurrence, incidence of metastasis, and mortality rates (1). Treatment options are limited since the hormonal therapy as well as HER-2 inhibitors typically used for other breast cancer types are ineffective. Due to the lack of a targeted therapy of choice, chemotherapy remains as the standard therapeutic approach for the treatment of TNBC patients (2,3). Despite the aggressive nature of TNBC, 20% of patients present a pathologic complete response (pCR) after chemotherapy (4). Paradoxically, despite a higher response rate to chemotherapy than other breast cancer subtypes, TNBC patients present a poor prognosis since they tend to suffer early recurrences leading to metastatic disease (5).

The early relapses observed for TNBC patients are caused by the resistance to chemotherapeutic agents, which translates into a low overall survival (6). Mechanistically, chemoresistance can be divided into primary and acquired resistance. Primary resistance is caused by an innate lack of therapeutic response, whereas acquired resistance arises during the course of therapy. The mechanisms of therapeutic resistance in breast cancer are diverse and a non-exhaustive list include: the overexpression of efflux membrane proteins as ATP-binding cassette (ABC) transporters, the establishment of feedback loops of signaling pathways, the upregulation of microRNAs, the overexpression of cell detoxifying enzymes like aldehyde dehydrogenase (ALDH), the upregulation of DNA repair mechanisms such as homologous recombination (HR) to remove the drug induced DNA lesions, an enhanced tumor cell plasticity mediated by EMT and stemness, as well as the hijack of the tumor microenvironment (7). Different studies also revealed the link between autophagy and therapeutic resistance (8,9). Autophagy is a catabolic pathway with a crucial role in the degradation and recycling of proteins and cellular components (10). Under normal conditions, cells utilize autophagy to maintain homeostasis by performing a quality-control of cell contents and eliminating old proteins and damaged organelles (11). However, in cancer cells the mechanism of autophagy is more complex. On one hand, it suppresses tumorigenesis by inhibiting cancer-cell survival and inducing autophagic cell death. On the other hand, it can also facilitate tumorigenesis by promoting tumor growth generally in advanced cancer. During therapeutic treatment, autophagy has been reported to act as a protective mechanism likely to participate in the development of acquired resistance (12). This dual role of autophagy is still controversial and several studies suggest that it is highly dependent on the tumor type, tumor stage and drug treatment (13).

Wang *et al*. (14) recently reported in this journal a study on the role of the eukaryotic elongation factor 2 kinase (eEF2K) as a modulator of autophagy in the

chemoresistance to paclitaxel in TNBC cells. eEF2K is a protein kinase that regulates the elongation stage of protein synthesis by phosphorylating and inhibiting eEF2, a protein that facilitates the movement of ribosomes along mRNA during protein synthesis (15). The authors used TNBC cell lines to model the resistance to paclitaxel, and then compared the effect of inhibiting autophagy and silencing eEF2K in sensitive versus resistant cells. The manuscript describes how the inhibition of autophagy and the ablation of the expression of eEF2K affect cell viability, tumor formation by 3D-culture and invasion in the context of paclitaxel resistance. The results reported in the manuscript show that autophagic flux was elevated in chemoresistant TNBC cells. The inhibition with chloroquine (CQ), a commonly used autophagy inhibitor, greatly reduced the half maximal inhibitory concentration (IC50) of paclitaxel in resistant cells, validating an important role of autophagy in chemoresistance. Moreover, using 3D models and Transwell assays, the authors confirmed that CQ treatment markedly reduced tumor growth and invasion of resistant cells, suggesting that autophagy could also mediate the aggressive tumor behavior of resistant tumors. Interestingly, they observed that the silencing of eEF2K suppressed the autophagic flux and caused a decreased viability and invasive behavior in the resistant cell lines. Based on these results, the authors suggest that the induction of autophagy during the response to paclitaxel may contribute to the maintenance of chemoresistance. Therefore, silencing eEF2K would revert the chemoresistance in resistant tumor cells. Moreover, the authors evaluated the potential of LC3 and eEF2K as prognostic biomarkers in TNBC patients with residual disease after neoadjuvant chemotherapy using paclitaxel and carboplatin. Specifically, analysis of eEF2K expression by IHC in samples from 222 patients showed that eEF2K positivity was associated with poor survival, and that correlation was stronger in patients with TNBC. The authors suggest that the positive expression of LC3 and eEF2K could identify resistant clones in TNBC tumors. Based on the combination of LC3 and eEF2K expression, they classified TNBC patients into subgroups with different levels of chemoresistance and relapse risk. In conclusion, all these data suggest a role of eEF2K as a regulator of autophagy, and a potential new target for the treatment of refractory TNBC tumors.

The results presented in the Wang *et al*. paper represent an interesting approach to try to overcome drug resistance in TNBC. Abundant data *in vitro* and *in vivo* supports the idea that autophagy facilitates the acquired resistance of tumor cells to chemotherapy (16). Specifically, TNBC, the most aggressive and chemoresistant BC subtype, shows a high level of autophagy. For example, Lefort *et al*. showed an upregulation of autophagy in a large cohort of TNBC tumors (17). Particularly, they validated LCB3, a protein involved in autophagosome formation, as a marker of poor prognosis in TNBC, with no prognostic value for other BC subtypes. Chen *et al*. also demonstrated a crucial role of autophagy in regulating the metastatic potential of TNBC (18). They showed that the yes-associated protein (YAP), which promotes autophagy, is upregulated in the TNBC cells compared to the ER+ cells. Furthermore, the inhibition of YAP prevented the migration and invasion of TNBC cells but it did not affect the mobility of ER+ cells. Taken all these data into consideration, the Wang *et al*. work confirms an important role of autophagy in the maintenance of aggressive tumor behavior and chemoresistance in TNBC. It also reinforces the idea that inhibiting autophagy could be a good strategy to re-sensitize tumor cells to chemotherapy.

Nevertheless, we believe that the data presented in this manuscript is still preliminary. First of all, there is an oversimplicity in the experimental models used to test the authors' hypothesis. We strongly believe that an *in vivo* model of the eEF2K knockdown would allow to analyze the therapeutic strategy proposed by the authors in a more physiological setting. Additionally, further validation in animal models is needed to understand the effect of the eEF2K knockdown in combination with classical cytotoxic agents used in TNBC. Furthermore, due to the complexity of autophagy, future studies would also benefit from comparing different approaches targeting autophagy. The authors demonstrated the effects of the inhibition of autophagy using either CQ or the ablation of expression of eEF2K, but a comparison between the two strategies is lacking. There is also evidence suggesting a dual role of eEF2K in cancer, both promoting cancer survival and tumor growth, and impeding tumorigenesis (19). For example, Xie *et al*. demonstrated that eEF2K suppressed tumorigenesis in colon cancer cells (20). Specifically, they observed that LC3-II levels increased and LC3 dots accumulated in cells when eEF2k was knocked down, suggesting a negative regulation of autophagy by eEF2k. This evidence contradicts the pro-autophagic effect observed in TNBC by Wang *et al*., highlighting the need for more work to understand the best approach to target eEF2K.

The use of autophagy inhibitors to treat cancer was very popular a decade ago. Several phase I/II cancer

clinical trials involving chloroquine (CQ) and its derivative hydroxychloroquine (HCQ), and aimed at treating different cancer types were done. CQ and HCQ block a critical step in autophagy by blocking autophagosome fusion and degradation. Despite the great effort, the clinical trials resulted into meek survival benefits for patients, and efforts to target autophagy faded. The reasons behind the lack of success include the fact that the effect of CQ and HCQ are not fully autophagy-specific. For example, a recent study by Maycotte *et al*. (21) reported that CQ could sensitize breast cancer cells to chemotherapy independently of autophagy inhibition. However, new approaches aimed at targeting autophagy are emerging including the use of inhibitors of ULK1, a protein kinase that regulates autophagosome biogenesis, or the inhibitor of VPS34, a protein implicated in diverse cellular processes, including endocytosis and autophagy (22). Besides, the Amaravadi's group reported an effective compound, called CD661, capable of deacidifying the lysosome and inhibiting autophagy significantly better than HCQ (23). According to the new efforts to tackle autophagy in cancer, we think that Wang *et al*. work could provide an additional approach to inhibit autophagy by targeting eEF2K. The role of eEF2K in controlling autophagy is well demonstrated (24) and its inhibition, particularly using specific kinase inhibitors, could be a novel strategy for the treatment of TNBC. We believe the author's findings present an interesting avenue to better understand the acquired resistance to treatment in TNBC that warrants further exploration.

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Footnote

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