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Is targeting autophagy a promising lead to unveil the cloak of invisibility in pancreatic cancer?

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Abstract

Pancreatic ductal adenocarcinoma PDAC is considered as one of the less immunogenic solid tumor types. Pancreatic tumors are also known to present a high autophagy flux which supports tumor progression. In PDAC, autophagy was recently described as a tumor-intrinsic immune escape process during tumor development by sequestration of Major Histocompatibility Complex class I (MHC-I) inside the PDAC cells. We comment this discovery and discuss the implications on how to limit immune escape in patients and how to improve immunotherapy efficiency.

Currently, pancreatic adenocarcinoma is the most frequent pancreatic cancer with a poor prognosis, an important lethality, and a 5-year overall survival less than 5%. The development of some therapeutic solutions like targeted therapies are promising [1]. However, it is still important to understand this morbid pathology to improve the treatment, because PDAC is predicted to be the second leading cause of death in Western countries [2].

Context

The use of immunotherapies improves the global patient survival in various solid cancers but fails in pancreatic cancer; this remains poorly understood. The PDAC microenvironment is enriched in immunosuppressive cells like myeloid-derived suppressor cells, macrophage and regulatory T cells. All these events contribute to render PDAC tumor microenvironment supportive of immune evasion [3].

Pancreatic tumor microenvironment is very dense, with a high level of extracellular matrix proteins that limit oxygen biodisponibility within the tumor. Pancreatic tumors are considered as a hypoxic microenvironment. Hypoxia can induce autophagy especially by hypoxia-inducible factor 1 α (HIF-1 α) [4,5]. To compensate with lack of nutrient availability, PDAC cells degrade and recycle cytoplasmic components to survive in stress conditions using macroautophagy process. Some studies have already demonstrated the importance of this catabolic process to maintain PDAC tumor growth [6], and confirmed that macroautophagy is the type of autophagy enriched in PDAC tumor. This process is regulated by a panel of ATG proteins that induces selective autophagy vesicles and requires cargo protein to sequester targeted proteins in these autophagosomes, that are then fused with lysosome leading to protein degradation [7].

Even if, autophagy and immunosuppression seem to play separately an important role in PDAC cancer progression, the use of autophagy inhibitor to starve the tumor or the use of immunotherapy to reshape the immune microenvironment in clinical trial did not show the expected effect. It is important to understand why these therapies are not that effective in PDAC, possibly through a specific process of immune escape. This subject was addressed by Yamamoto and collaborators in an article published in *Nature* in April 2020 [8].

MHC-I expression is modified in PDAC cells

They used an important diversity of human cell lines, including human PDAC cell lines (PaTu8988T, KP4, Mia PaCa-2, PANC0203, Panc1, AsPc1, HupT3) (that corresponds to commonly used or rarely used cell lines), that were compared with HPDE (an immortalized ductal pancreatic cell line). First, MHC-

I expression was heterogeneous through all the cell lines studied. Yamamoto and collaborators showed that MHC-I was more expressed inside the cell in PDAC human cell lines compared to normal cell line (HPDE). They showed that MHC-I was co-localized with LAMP1 (lysosomal protein) and LC3-B (autophagosome protein). In conclusion, in the PDAC cells, the MHC-I was relocated inside the cell, at the level of the lysosome or in autophagosome rather than on the cell surface, as observed in normal HPDE cells. The same observation was confirmed on patient samples with PDAC.

Autophagy inhibition relocalizes MHC-I expression at the plasma membrane

Macroautophagy rate also called flux is increased in PDAC. To study the impact of autophagy on the MHC-I expression, the authors inhibited the autophagy process by different approaches, namely ATG3 knock-down, ATG7 knock-down or lysosomal inhibition with Bafilomycin A1, in different human PDAC cell lines. Autophagy inhibition in PDAC maintained MHC-I expression at the plasma membrane. Macroautophagy is thus a process involved in MHC-I trafficking between plasma membrane and lysosome and regulates MHC-I bio-disponibility.

Macroautophagy is a targeted process and requires cargo proteins to control selective protein degradation. Yamamoto *et al.* therefore studied the signalling pathway enabling targeted autophagy of MHC-I. They used the proximity biotinylation assay fused to MHC-I protein in order to highlight the cargo protein involved. After an analysis of some potential cargo proteins, they demonstrated the implication of NBR1. Specific NBR1 knock-down showed the same effect than autophagy inhibitor on MHC-I expression level and localization. MHC-I expression level and localization are regulated by the autophagy cargo protein NBR1.

Autophagy inhibition remodels immunogenicity in the tumor microenvironment

To evaluate the impact of maintained MHC-I expression at plasma membrane by autophagy inhibition on antigen presentation, the authors used an elegant strategy to confound tumor-intrinsic and tumor-extrinsic action. They engineered a PDAC murine cell line to express after induction by doxycycline a dominant negative ATG4B (that is necessary for the elongation of autophagic vesicles) in presence of OVA neoantigene (that are presented by MHC-I at plasma membrane) (**Figure 1A**). In these cells, doxycycline induced an increase: - in MHC-I expression at the plasma membrane, - in antigen presentation. The co-culture experiment with OVA specific CD8+ T cells and PDAC cell pre-treated with doxycycline showed an increase in T cell proliferation and cytokine expression (IFN γ , TNF). Autophagy inhibition prevents immune escape and promotes T cell antitumor activity.

To study the in vivo effect of autophagy inhibition, Yamamoto and collaborators transplanted orthotopically the cell line described above. Genetic tumor-intrinsic autophagy inhibition favored smaller tumor with high MCH-I expression at the plasma membrane and increased immune CD8+ T cell infiltration, associated with a reduction in myeloid-derived suppressor cell infiltration. These effects were also observed when cells were intrasplenically injected into syngeneic mice (C57BL/6) as a model of liver metastasis; autophagy inhibition in tumor cells led to a lower metastasis burden.

In PDAC cells from genetically engineered mouse models, the authors found that autophagic flux was heterogeneous. PDAC cells with low autophagy flux presented a decrease in their clonogenic capacity, a reduction of tumor mass when injected in vivo models associated with an increase in CD8+ T cells infiltration, mimicking a context of autophagy inhibition. Autophagic flux could therefore represent a reflection of tumor immunogenicity. It could be important to determine the autophagic flux within the tumor in Humans to adapt the therapeutic options and increase the impact of immunotherapies.

Autophagy inhibition in addition with immunotherapies a new therapeutic option for PDAC?

Finally, to determine the therapeutic benefits of autophagy inhibition on immunotherapy in PDAC treatment the authors treated PDAC mice model with chloroquine, a systemic autophagy inhibitor, in combination with anti-PD1 alone or in combination with anti-CTLA4 antibody. The combination had an

interesting impact particularly on tumor growth: it decreased autophagic flux, increased the tumor immunogenicity by increasing MHC-I expression at the plasma membrane and increasing CD8+ T cell infiltration. In conclusion, the authors demonstrate that combination between immunotherapy (anti-PD1 and anti-CTLA4) with autophagy inhibitor could be promising therapy in PDAC treatment.

The therapeutic use of a combination of autophagy inhibitor with immunotherapies seems to be a good option according to the data published in the article by Yamamoto *et al.* (**Figure 1A**). Nevertheless, the use of chloroquine or derived molecule to inhibit autophagy in a systemic way may not be the best solution because of risks for cardiotoxicity and particularly on patients weakened by cancer.

Perspectives: use of class III PI3K inhibitors as modulators of autophagy, impact on other targeted therapies that modulate autophagy flux

The PI3K class III is composed by one enzyme, Vacuolar Protein Sorting 34 (Vps 34). It phosphorylates phosphatidylinositol into phosphatidylinositol-1-phosphate and allows the recruitment of an autophagy-inducing complex containing Beclin-1, Atg14 and other autophagy-related (Atg) proteins to the site of autophagosome permitting its elongation [9]. After autophagosome maturation, Vps34 helps to fusion with lysosome to degrade and recycle its content to fuel cell catabolism.

Another study by Noman *et al.* published in *Science advances* in April 2020 confirmed the importance to inhibit autophagy with Vps34 inhibitor in addition with immunotherapy [10]. In different cancer types such as melanoma or colorectal cancer, they also showed an increase of anti-tumor immunity within the tumor when they inhibited autophagy. In the context of treatment with immunotherapy such as anti-PD1 or anti-PD-L1 treatments, the combination with Vps34 inhibitor improved the immunotherapy efficiency. However, each cancer types present different mechanisms of autophagic induction as well as different adaptive responses to autophagic flux disruption. Thus, it remains to be tested in PDAC patients, whether Vps34 could also be a target of interest. Balance with toxicity to liver or cardiac function [11,12] is another parameter to take into account.

With regards to PI3K signaling and autophagic flux, some critical questions are remaining. Indeed, the PI3K class I is composed by four enzymes; α , β , γ , δ . They catalyze the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3) and permit the stimulation of the PI3K/Akt/mTOR axis [13]. PI3K α is the major activator of mTORC1 activity. When mTORC1 is activated, it phosphorylates the protein ATG 13 (Autophagy-related gene 13) that inhibits autophagy [14]. In 90% of PDAC cells, we observe a mutation of the GTPase KRAS which hyperactivates the axis PI3K/Akt/mTOR through PI3K class I alpha [15]. How can we explain that in one hand pancreatic cancer cells present a high level of autophagy [16,17] and in the other hand, the axis PI3K α /Akt/mTORC1 that inhibits autophagy is overstimulated in pancreatic cell? In our opinion, understanding the mechanisms of this paradigm can be interesting to improve our knowledge in autophagy in pancreas cancer cell to find other way to inhibit it (**Figure 1B**).

To conclude, high level of autophagy and immunosuppressive micro-environment are two hallmarks of pancreatic cancer. In this paper, Yamamoto *et al.* point the interest of investigating the underlying mechanism between autophagy and immunological cell state in pancreatic cancer. High level of autophagy is necessary for growth tumor and seems implicated in immune micro-environment protumoral state, by modulating the level expression of MHC-I at the membrane cancer cell.

Inhibiting autophagy in pancreatic ductal adenocarcinoma could avoid the growth tumor and increase the efficacy of immunotherapy. In this context, exploring the impact of PI3K enzymes on autophagy could be interesting for the future (**Box1**).

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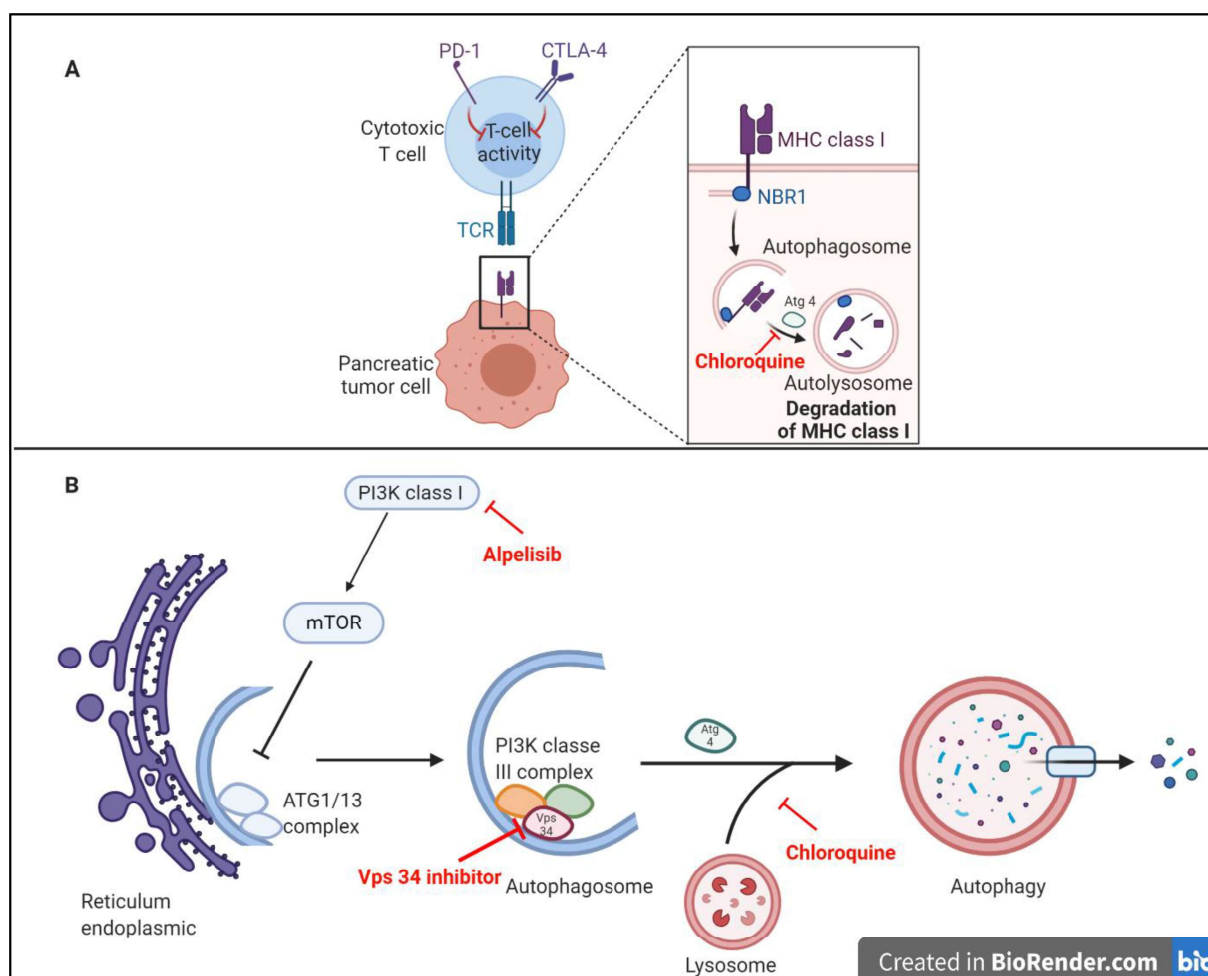
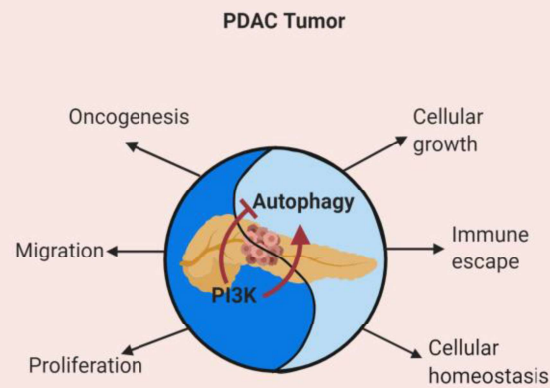


Figure 1: Autophagy, MHC-I degradation mechanism in pancreatic cancer cells

- A. MHC-I degradation by autophagy modulates the immunogenic state of PDAC cells
- B. PI3K enzymes are implicated in autophagosome synthesis: Stimulation of PI3K α /Akt/mTORC1 axis inhibits autophagosome synthesis while class III VPS34 promotes autophagy flux.

Box: PI3Ks and autophagy in PDAC.



- Class I PI3K (particularly PI3K α) is necessary for tumor growth and tumor dissemination. The activation of PI3K/Akt/mTOR pathway induces ATG13 phosphorylation by activated mTOR. This phosphorylation on ATG13 inhibits autophagic process [14].
- Class III PI3K, Vps34, allows the formation of autophagy inducing complex and is responsible of the high autophagic flux in PDAC tumor [9].
- Phosphoinositide phosphorylation dynamic is regulated by the different class of PI3K, they support both tumoral cell migration and proliferation while activating autophagy to maintain growth, cellular homeostasis, and immune escape mechanisms.
- One major question remains: **how this dual function of PI3K is coordinated to support tumor growth and progression?**

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