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AUTHOR'S VIEWS



## Starving PTEN-deficient prostate cancer cells thrive under nutrient stress by scavenging corpses for their supper

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### ABSTRACT

Our recent work demonstrates that inactivating mutations in phosphatase and tensin homolog (*PTEN*) are sufficient to drive macropinocytosis in the context of AMP-activated protein kinase (AMPK) activation. Given that blocking macropinocytosis limits *PTEN*-deficient prostate tumor growth, AMPK or phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitors could have therapeutic value in castration-resistant prostate cancer patients, particularly when used in combination with standard of care therapies.

**Abbreviations:** ATG5: autophagy related 5; NHE: Na<sup>+</sup>/H<sup>+</sup> exchanger; PAK1: p21-activated kinase 1; PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase; PIP<sub>3</sub>: phosphatidylinositol (3,4,5)-trisphosphate; PIP<sub>2</sub>: phosphatidylinositol (4,5)-bisphosphate; RAC1: Rac family small GTPase 1

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Inadequate perfusion makes it difficult for tumor cells to acquire sufficient nutrients to sustain their non-homeostatic, oncogene-driven proliferation.<sup>1</sup> Macropinocytosis, an actin-dependent form of bulk endocytosis, allows Kirsten rat sarcoma viral oncogene homolog (KRAS) driven pancreatic ductal adenocarcinomas (PDAC) to proliferate despite amino acid depletion.<sup>2,3</sup> Phosphatidylinositol<sup>3-5</sup>-trisphosphate (P<sup>3-5</sup>P<sub>3</sub>; best known as PIP<sub>3</sub>) generated by class 1 phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) is necessary for macropinosome formation.<sup>4</sup> Tumor suppressor phosphatase and tensin homolog (*PTEN*), which is deleted or inactivated in many cancer classes, opposes PI3K by converting PIP<sub>3</sub> to phosphatidylinositol 4,5-bisphosphate (P<sup>4,5</sup>P<sub>2</sub>; best known as PIP<sub>2</sub>). Our finding that macropinocytosis also supports the growth of *PTEN*-deficient prostate cancer cells in vitro and in vivo demonstrates that the potential impact of therapeutic macropinocytosis inhibitors could be much greater than previously appreciated.<sup>5</sup>

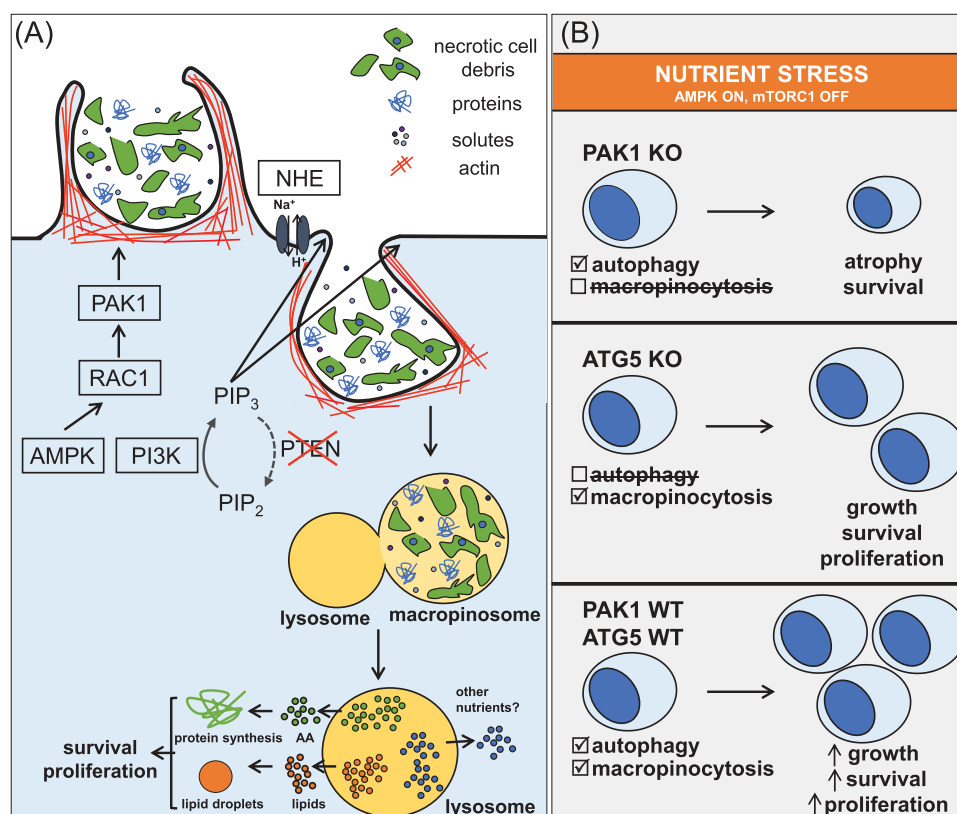
### PTEN loss and AMPK activation promote macropinosome formation

*PTEN* null or deficient prostate cancer cell lines, patient-derived organoids, and autochthonous, patient-derived xenograft, and isograft prostate tumors exhibit macropinocytosis that is blocked by class I PI3K inhibitors or *PTEN* reconstitution.<sup>5</sup> An unexpected role for AMP-activated protein kinase (AMPK) in promoting macropinocytosis was also uncovered. AMPK promotes macropinosome formation by activating Rac family small GTPase 1 (RAC1) that coordinates the actin remodeling necessary for membrane ruffling. Although AMPK activated RAC1 in *PTEN*-replete cells, macropinosomes only formed if *PTEN* was inactivated in keeping with previous studies showing that PIP<sub>3</sub> is necessary for macropinosome closure (Figure 1A). These

mechanistic studies add to a growing body of literature suggesting that AMPK inhibition makes more therapeutic sense than activation in established prostate tumors.<sup>6</sup> AMPK inhibitors would undermine not only macropinocytosis, but also autophagy. It will be important to conduct genetic experiments where AMPK is inhibited in established tumors to evaluate this hypothesis.

### Targeting macropinocytosis for cancer therapy

To date, no specific macropinocytosis inhibitors have been identified. The most commonly used macropinocytosis inhibitor, EIPA, targets Na<sup>+</sup>/H<sup>+</sup> exchangers (NHE) resulting in pleiotropic antitumor effects that could make EIPA an excellent therapeutic.<sup>7</sup> As systemic administration of EIPA blocks macropinocytosis and significantly reduces prostate tumor growth, even producing some regressions,<sup>5</sup> the conventional wisdom that EIPA's pharmacologic properties preclude its use in the clinic merits reevaluation. More potent and selective NHE inhibitors could also be effective. While RAC1 and p21-activated kinase 1 (PAK1) inhibitors block macropinocytosis and are valuable as tool compounds, viable clinical candidates are lacking. In *PTEN* null MEFs, inhibition of both  $\alpha$  and  $\beta$  isoforms of PI3K was necessary to block macropinocytosis. Combining  $\alpha$  and  $\beta$  isoform-selective class 1 PI3K inhibitors that are in late-stage clinical trials may be a better approach than deploying pan-PI3K inhibitors which have been plagued by on-target toxicities. Given our finding that macropinocytosis, but not autophagy, drives growth in nutrient-limiting conditions, the beneficial effects of lysosomal inhibitors like chloroquine may be derived more from the inhibition of macropinocytosis than from blocking autophagy (Figure 1B).



**Figure 1.** Macropinocytosis allows PTEN-deficient prostate cancer cells to proliferate in nutrient-limiting conditions. (A) Phosphatase and tensin homology (*PTEN*) loss and AMP-activated protein kinase (AMPK) activation is sufficient to induce macropinosome formation. Macropinocytosis allows non-selective uptake of necrotic cell debris, proteins, and extracellular fluid. Lysosomal catabolism of the macromolecules present in macropinosomes provides anabolic substrates. Boxed proteins are potential targets for pleiotropic macropinocytosis inhibitors. (B) Recycling intracellular components via cell autonomous autophagy fuels survival, but not growth, in nutrient-limiting conditions. Because macropinocytosis provides access to extracellular macromolecules, it can support both survival and proliferation.

## Necrocytosis: a new tumor-microenvironment interaction

Scavenging extracellular matrix proteins and albumin can provide amino acids, but poor perfusion is also likely to compromise tumor cell access to lipids, sugars and nucleotides. Our demonstration that macropinocytotic prostate cancer and PDAC cell lines can use necrotic cell corpses to fuel proliferation in amino acid- and glucose-deficient medium implies that this scavenging pathway has more to offer than just amino acids. Macropinocytosis of necrotic cell debris, but not albumin, increased lipid droplet content in starved prostate cancer cells suggesting that lipids are also harvested from cellular corpses. Macropinocytotic consumption of necrotic corpses (necrocytosis) also affords unique opportunities for isotopic labeling studies, allowing us to demonstrate that even in nutrient-replete medium 15–25% of the amino acids in newly synthesized proteins were derived from macropinocytosis. Advanced, drug resistant solid tumors often contain necrotic regions,<sup>8</sup> and cytotoxic standard of care therapies may generate cellular corpses that paradoxically fuel the growth of the surviving cells. Thus, macropinocytosis inhibitors may be particularly effective as part of combination therapies. Combining  $\alpha$  and  $\beta$  isoform-selective class 1 PI3K inhibitors with the androgen receptor inhibitor enzalutamide produced regressions in pre-clinical, PTEN-deficient prostate

cancer models;<sup>9</sup> macropinocytosis inhibition could contribute to the enhanced activity of this combination. While the pan-PI3K inhibitor BKM120 has been tested in combination with enzalutamide in a clinical trial,<sup>10</sup> enzalutamide substantially increased the metabolism and clearance of BKM120, and it was not clear whether PI3K was inhibited in patients receiving the combination. Patients were also not selected for mutations that would drive macropinocytosis (e.g. *PTEN* inactivation or loss). Thus, whether inhibiting macropinocytosis will enhance androgen deprivation therapy or overcome resistance in castration-resistant prostate cancer patients remains an open question.

In conclusion, our study suggests that the contribution of macropinocytosis to tumor anabolism has been significantly underestimated. Several drugs in the clinical pipeline could be used to block macropinocytosis in patient tumors and will likely be most effective when used in combination with existing therapies. Because these agents all have pleiotropic actions, more selective approaches will be required to dissect the contribution macropinocytosis makes to tumor growth and metastasis.

## Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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