IN THE SPOTLIGHT

The Golgi: Keeping It Unapologetically Basic

Nathan P. Ward and Gina M. DeNicola

Summary: Tumor cells maintain a reverse pH gradient relative to normal cells, conferring cell-intrinsic and cell-extrinsic benefits that sustain tumor growth. In this issue of *Cancer Discovery*, Galenkamp and colleagues reveal that NHE7 mediates acidification of the *trans*-Golgi network in pancreatic ductal adenocarcinoma, which is critical for the maintenance of cytosolic pH and consequently tumor growth.

See related article by Galenkamp et al., p. 822 (6).

The acidification of the tumor microenvironment is increasingly appreciated as a major contributor to disease progression (1). The development of tumor acidosis is attributed to the genetic and environmental drivers of hallmark Warburg metabolism, in which cancer cells exhibit enhanced glycolytic flux even in the presence of adequate oxygen. This increased glycolytic metabolism supports the energetic and biosynthetic demands of rapidly proliferating tumors. However, a by-product of this activity is the production of copious H^+ ions from the rapid hydrolysis of ATP (1). This imposes a potential restraint on the sustainability of Warburg metabolism, as several glycolytic enzymes function optimally at an alkaline pH (2). In fact, tumor cells maintain an alkaline intracellular pH (pH_i) relative to untransformed cells that permits unbridled proliferation (2). This is achieved through the upregulation of plasma membrane transport systems that mediate H^+ efflux. Although the proton-translocating family of monocarboxylate transporters (MCT) that couple the export of H^+ ions with monocarboxylates, including lactate, have gained the most attention, the plasma membrane Na^+/H^+ exchanger (NHE1) also plays a significant role in maintaining alkaline pH_i in cancer and confers significant growth advantages to tumors (3).

Though acidification of the microenvironment is often specifically linked to increased glycolytic metabolism, it is in fact a consequence of the totality of the metabolic rewiring exhibited by tumors. Many tumors exhibit an accompanying increase in mitochondrial metabolism associated with substantial CO_2 release (1). This further enhances extracellular acidification through stimulation of carbonic anhydrases, which are concomitantly upregulated with MCTs and support their function (4). In concert, the extensive metabolism of tumors establishes a considerable pH gradient across the plasma membrane that enhances the fitness of tumor cells at the expense of other stromal compartments. Although acidification of the microenvironment elicits extrinsic benefits in immunosuppression and extracellular matrix degradation, the maintenance of an alkaline pH_i is also intrinsically advantageous (1). The loss of extracellular rigidity potentiates the increased motility of tumor cells with alkaline pH_i due to the enhanced activity of the actin remodeling protein cofilin (2). In addition to permitting the metabolism required for biomass accumulation, alkaline pHi also facilitates G_2–M progression and DNA synthesis in support of the rapid proliferation of cancer cells (3). Furthermore, alkaline pH confers resistance to apoptosis, which presents with cellular acidification, a requisite for caspase activation (2).

As described, much of the effort to elucidate the etiology of tumor acidification and the maintenance of alkaline pH_i in tumor cells has focused on the influence of plasma membrane H^+ transporters. However, recent evidence suggests that pH_i is also regulated by organellar sequestration of H^+ ions via these H^+ transporters (5). In this issue of *Cancer Discovery*, Galenkamp and colleagues elegantly describe a role for the NHE family member NHE7 in maintaining alkaline pH_i in pancreatic ductal adenocarcinoma (PDAC) through support of *trans*-Golgi network (TGN) acidification (6). Through an extensive evaluation of available patient data, the authors identified that elevated NHE7 expression correlated with poor prognosis. To investigate the importance of NHE7 in PDAC, the authors disrupted NHE7 expression in a panel of PDAC cell lines and found that loss of NHE7 blunted proliferative capacity due to enhanced cell death but had no effect on untransformed controls. Given that NHE7 can associate with various cellular compartments (7), the authors utilized immunofluorescence to reveal that NHE7 localized to the TGN in PDAC cells. They next employed a pair of TGN-targeted probes reliant on EGFP (pH-sensitive) and mCHERRY (pH-insensitive) fluorescence to establish that NHE7 activity regulates luminal acidification of the TGN. Intriguingly, although NHE7 deficiency did disrupt protein sialylation, preceding glycosylation events (i.e., *N*-glycosylation) were not affected, nor was protein trafficking to the plasma membrane. This diminished sialylation was not relevant to the decrease in cell viability associated with NHE7 loss, suggesting an alternative function for NHE7 within the TGN.

Given the observation that NHE7 mediates proton translocation from the cytosol to the TGN in PDAC cells, Galenkamp and colleagues hypothesized that loss of NHE7 would

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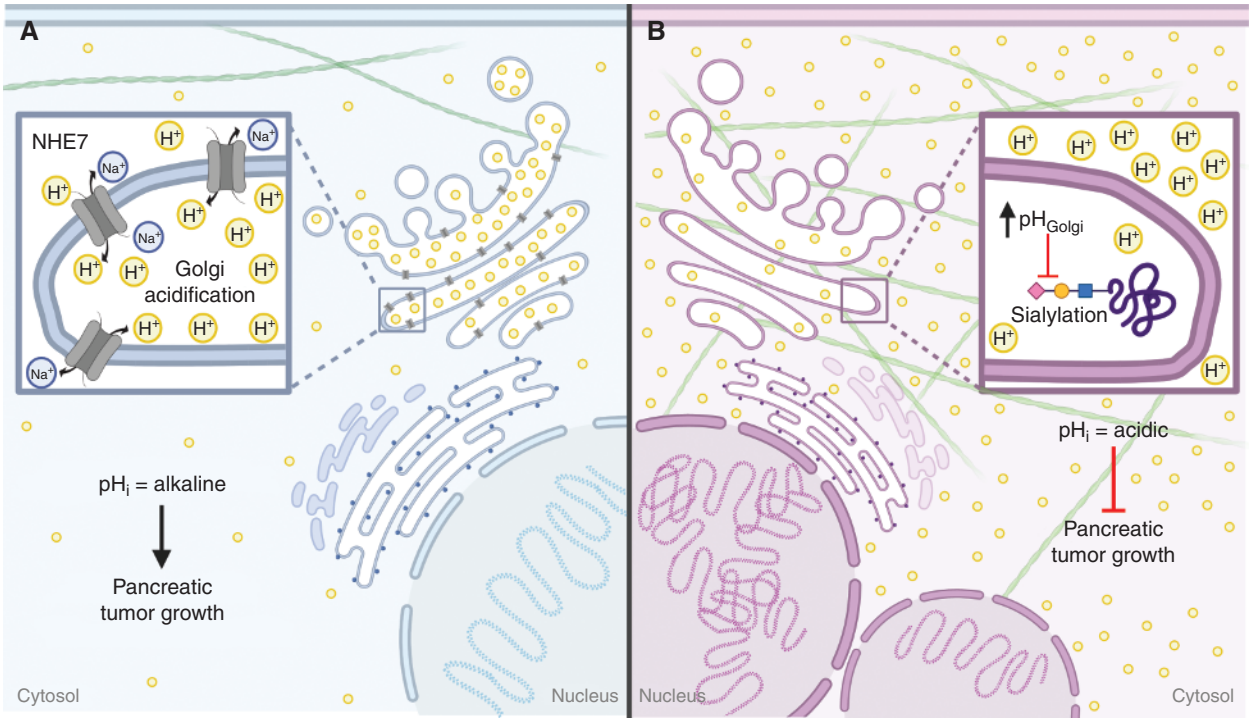

Figure 1. NHE7 supports PDAC tumor growth through maintenance of intracellular pH. **A,** Considerable metabolic acid in the form of H^+ is generated as a by-product of ATP hydrolysis, which supports the biosynthetic metabolism and aberrant signaling associated with tumorigenesis. NHE7, a Golgi-associated H^+/Na^+ exchanger, mediates the sequestration of cytosolic H^+ in the Golgi lumen and the subsequent acidification of the trans-Golgi network. This activity maintains an alkaline pH_i that is permissive for PDAC growth. **B,** Loss of NHE7 in PDAC cells elicits a significant alkalinization of the Golgi and a corresponding increase in pH_i that is unsustainable, leading to loss of PDAC cell viability and a restriction of tumor growth. Disruption of Golgi acidification inhibits protein sialylation but does not restrict protein trafficking. Rather, a more detrimental consequence of NHE7 loss is a dysregulation of the actin cytoskeleton due in part to an inhibition of the actin-severing protein cofilin. This manifests with an accumulation of actin stress fibers (green) and significant nuclear abnormalities such as multinucleation and an increase in DNA content. Blue square, *N*-acetylglucosamine; yellow circle, galactose; pink triangle, sialic acid.

have a detrimental effect on pH_i. Indeed, use of an intracellular pH indicator revealed that loss of NHE7 significantly reduced pH_i in PDAC cells but not in untransformed controls. Further, the authors show that loss of NHE7 expression blunts the capacity of PDAC cells to recover from acid loading, suggesting that NHE7 permits the TGN to serve as a H^+ sink.

Enhanced cell motility with an alkaline pH_i is due to enhanced capacity for actin remodeling proteins to alter the cytoskeleton (2). Thus, the authors surmised that increased pH_i would have detrimental effects on the actin cytoskeleton. Indeed, NHE7 loss promoted pH-dependent cofilin phosphorylation and diminished actin depolymerization, leading to an accumulation of actin stress fibers. Consistent with the role of stress fiber disassembly in cytokinesis (8), NHE7 deficiency promoted the nuclear deformation, multinucleation, and increased DNA content. Importantly, pharmacologic resolution of the actin stress fibers in NHE7-deficient cells rescued cell viability. This suggests that NHE7 regulation of TGN acidification plays a homeostatic function in the maintenance of pH_i, such as serving as a reservoir for the substantial H^+ produced as a consequence of enhanced tumor metabolism (Fig. 1). To confirm a relevance for NHE7 activity in PDAC tumors, the authors performed a series of xenograft experiments. Doxycycline-mediated knockdown of NHE7 in established xenografts resulted in tumor growth arrest or regression, further supporting a protumorigenic function for NHE7 in PDAC.

The work of Galenkamp and colleagues reveals NHE7 as a critical regulator of pH_i in PDAC through maintenance of TGN acidification. Moreover, this work highlights how disruption of organellar control of pH_i is detrimental to cell viability and tumor growth, further broadening our understanding of pH regulation in cancer. Given the discrete susceptibility of PDAC cells to NHE7 loss demonstrated herein, the authors have potentially uncovered a unique vulnerability that can be exploited clinically for PDAC. The delayed clinical detection and underlying biology of PDAC imposes an urgent need to identify robust therapeutic targets (9). Substantial effort has been expended in the hope of disrupting the pH gradient in tumors therapeutically (1). Similar to other targeted therapies, agents targeting plasma membrane H^+ transporters are often rendered inefficacious due to the redundancy of these systems (10). The specific reliance of PDAC cells on NHE7 suggests there may be less plasticity in organellar H^+ transporters, making tumors more susceptible to the disruption of intracellular H^+ transport. Regardless, this study demonstrates the potential utility of targeting NHE7 and the need for further investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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