REVIEW ARTICLE

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Effects of Tumor Microenvironment Acidification on Progression of Pancreatic Ductal Adenocarcinoma: A Review

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive and devastating disease, which is characterized by invasiveness, rapid progression and profound resistance to treatment. It has been best characterized that tumor microenvironment such as hypoxia and nutrient deprivation contributes to cancer progression; however, the role of tumor microenvironment acidification (TMA), a major feature of tumor tissue, has not been intensively studied. Interestingly, clinicopathological clues have recently unraveled that TMA is involved in promoting cancer progression although the exact signaling pathways is poorly understood. In PDAC, the TAM is tightly regulated by proton (H+) transporters and pumps. This review dissects and summarizes the roles of these H+-extruding regulators in facilitating PDAC progression.

Key words H+ transporters, H+ pumps, TMA, PDAC

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Introduction

PDAC is the most prevalent neoplastic disease of the pancreas responsible for more than 90% of all pancreatic malignancies [1], and is the third leading cause of cancer-related morbidity and mortality in both sexes, with a 5-year overall survival rate below 5% and a median survival of less than 6 months [2]. PDAC is associated with extremely poor prognosis, and the surgical resection followed by adjuvant chemotherapy drugs such as gemcitabine, erlotinib, FOLFIRINOX [3, 4] and/or a combination of nanoparticle albumin-bound-bound (nab)-paclitaxel and gemcitabine [5] is the only curative therapy currently applicable. Yet, it is still disappointing that most patients with advanced PDAC die within 12 months of diagnosis [6].

The pancreas is a complicated organ consisted of both exocrine glands (acinar and ductal cells secreting digestive enzymes into the intestinal lumen) and endocrine (α, β, δ, ε) glands, also known as the islets of Langerhans, which are responsible for secreting hormones into the blood stream [7]. Under certain extracellular stimuli such as tissue damage, stress conditions, or inflammatory factors, acinar cells can transdifferentiate into cells expressing specific ductal markers [8-10]. During acinar-to-ductal metaplasia (ADM), acinar cells acquire 'progenitor cell-like' properties that render them more susceptible to pro-oncogenic hits, such as activating mutations in the proto-oncogene Kirsten rat sarcoma virus (KRAS), eventually transforming them into pancreatic intra-epithelial neoplasias (PanINs). This transformation is generally considered as the initial step in PDAC development followed by sequential progression involving genetic hits in several tumor suppressor genes. Many studies have indicated that the gene encoding the proto-oncogenic GTPase KRAS as well as several tumor suppressor genes, consisting of tumor suppressor p53 (TP53), cyclin-dependent kinase inhibitor 2A (CDKN2A), and mothers against decapentaplegic homologue 4 (SMAD4), exhibit the most frequent alterations and/or mutations in PDAC [11, 12]. Besides, RAC-beta serine/threonine-protein kinase (AKT2) is frequently overexpressed [13, 14], and the activity of its upstream regulator phosphoinositide 3-kinase (PI3K) is often enhanced in PDAC, which leads to increased cancer cell survival [15, 16].

The PDAC tumor microenvironment (TME) is principally consisted of various cell types such as fibroblasts, endothelial cells, neurons, and infiltrating immune cells as well as the extracellular matrix (ECM) proteins such as cytokines, growth factors and blood vessels. The majority of the PDAC histology is desmoplasia derived from the stroma/desmoplastic reactions. The interactions amongst components in TME and the cancer cells play a central role in facilitating immune escape, tumor progression, and metastasis. The major characteristics of TME include hypoxia, nutrient deprivation and extracellular acidification that are thought to be the potential activators of cancer progression and metastasis. Whereas hypoxia and nutrient deprivation have been well-documented, TMA in PDAC has not been intensively studied. However, recent studies have confirmed that TAM is involved in initiating the early events of malignant transformation [17-20]; more crucially, promoting tumor progression and metastasis. In the context of TMA-mediated cancer progression, it is well known that invasiveness and metastasis of cancer cells are accelerated by a variety of extracellular proteases such as metalloproteinases (MMPs), thiol proteases, serine proteases and acid proteases, which are responsible for degrading the tumor barriers, creating ideal condition to favor tumor metastasis. Notably, the proteolytic activity of these enzymes could be optimized in the TMA.

Cancer cells exert aerobic glycolysis to generate energy and supply intermediates for macromolecule biosynthetic that are required for cell survival, differentiation and proliferation [21]. A common by-product generated by this metabolic pathway is lactate, which is converted from pyruvate by lactic acid dehydrogenase-A (LDHA) [22]. The cytosolically accumulated lactate is extruded into the TME via activated H+-linked monocarboxylate transporters (MCTs), subsequently acidifying TME [23]. Lactate activates vascular endothelial growth factor (VEGF) [24], transforming growth factor beta (TGFβ) [25], interleukin-1 (IL-1) [26] and HIF-1 [27]. Also, it is worth noting that glycolytic inactivation in cancer cells still facilitates acidification of the TME [28-30], indicating that the H+ efflux pathways might be regulated by other H+ extruders, in addition to MCTs, which include Na+/H+ exchangers (NHEs) [31, 32], sodium/bicarbonate transporters (NBCs) [33], V-type H+ ATPases [34, 35], and carbonic anhydrases (CAs) [36]. Therefore, addressing the question of why cancer cells, but not normal, non-transformed cells, can thrive in the TME is of utmost importance since therapies interfering with the TME might provide useful clinical strategies for patients with cancer. This review will summarize the specific roles of H+ transporters, pumps and channels in facilitating the PDAC development and progression.

MCTs

In the absence of oxidative phosphorylation, the glycolytic metabolism of cancer cells is initially facilitated by the uptake of glucose via glucose transporters (GLUTs), which belong to the solute carrier (SLC2A) family of transport proteins [37]. Lactate, the glycolytic end-product generated by PDAC cells, is extruded into the TME via the MCT1 [38]. MCTs are encoded by the solute carrier 16 (SLC16) family of genes. Among the 14 members of this family, MCT1/SLC16A1, MCT2/SLC16A7, MCT3/SCL16A8, and MCT4/SCL16A3 (hereafters referred to as MCTs) convey monocarboxylate ions together with H+ ions. In most cases, the lactate taken up by cancer cells derives from surrounding stromal cells, such as fibroblasts. The lactate/H+ MCT1 and MCT4 play a pivotal role in transferring energy through establishing a lactate-shuttle system. Under this condition, MCT1 favors cellular lactate-uptake, while MCT4 rather exports lactate [39]. In PDAC, Sukeda et al. previously reported that MCT4 that was expressed in cancer-associated fibroblasts (CAFs) tended to shorten overall and progression-free survival, whereas MCT1 expression was associated with prolonged survival and reduced lymph node metastasis [40], suggesting a multi-directional functionality of MCTs-mediated lactate secretion into TME upon facilitating development and progression of PDAC. However, De-Hai Wu et al. showed that miR-124-mediated MCT1 suppression abolished the glycolytic activity and altered intracellular acidification of PANC-1 cells, and more importantly, reduced the tumor phenotype in vitro and in vivo [41]. Besides, CD147, a membrane protein highly enriched on many human epithelial cancer cells, including PDAC, has recently been identified as a vital regulator of several membrane transporters, comprising MCT-1 and MCT-4 [42]. An important role of CD147 is to promote the synthesis of MMPs in CAFs [43, 44], suggesting that CD147 might be involved in altering the TME to favor invasiveness and metastasis. Indeed, knockdown of CD147 expression weakened tumorigenicity through inhibiting the lactate transport in both in vitro and in vivo models [42]. Furthermore, CD147 has also been associated with glutamine transport [45] and Ca2+ signaling [46], suggesting that CD147 might be a potent target for therapeutic treatments of patients with PDAC.

Alternatively, lactate discharging might be mediated through gap junctions that are connected by connexin channels. Previously Dovmark et al. demonstrated that Cx43 channels played a role as the crucial conduits for transmitting lactate from glycolytic PDAC cells into the neighboring cells, which triggers alkalization of recipient cells [47]. Markedly, cell-cell contact via this junctional

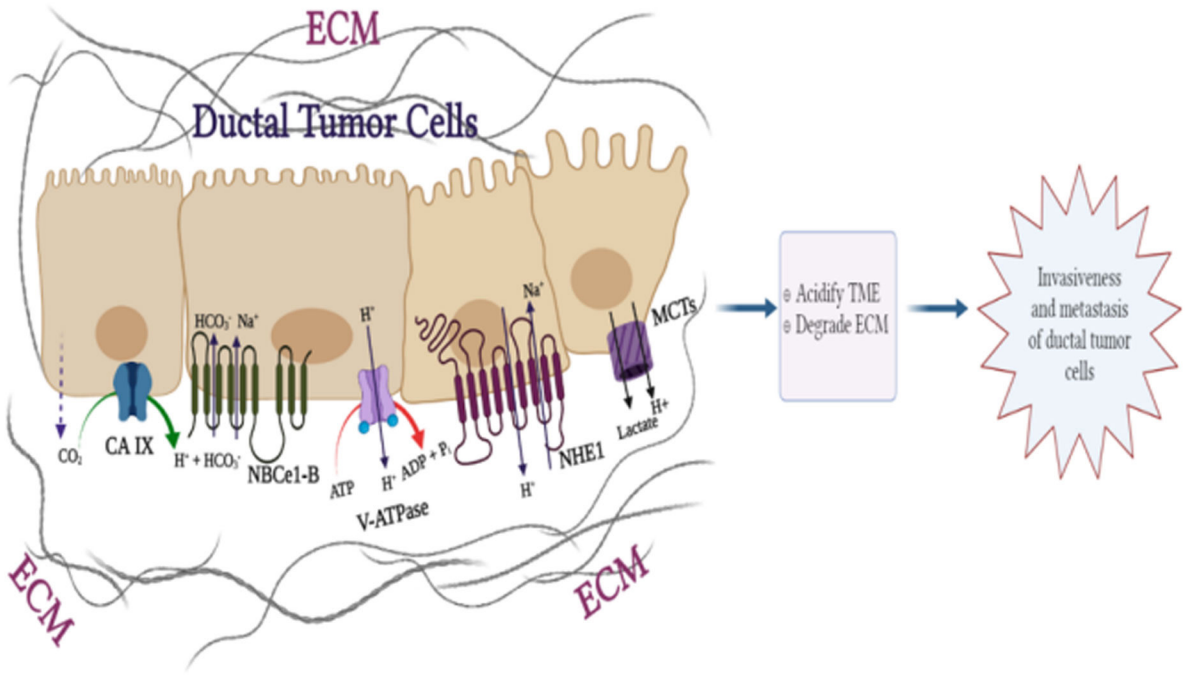

Figure 1. The possible mechanism underlying the TMA-mediated regulation of invasiveness and metastasis. The anaerobic glycolysis of ductal tumor cells enhances lactate production. Then, lactate is transported across plasma membrane via MCTs, mainly MCT-1 and -4 in a H+-dependent manner. In PDAC cells, NHE1, V-ATPases, and CA IX are frequently overexpressed to enhance the H+ transport into extracellular milieu, contributing to acidify the TME. Acidification of the TME subsequently provides the ideal milieu for enzymatic activity of MMPs to degrade the ECM, thereby facilitating the invasiveness and metastasis of PDAC cells.

flux is thermodynamically insensitive to TME, which possibly compensates for the TME-induced reduction of MCT activity. PDAC tumors are characterized by profoundly under-perfused regions requiring efficient mechanisms for discharging and transmitting lactate across clusters of PDAC cells [47], towards specific regions that possess the most favorable transmembrane gradient for MCT-assisted off-loading lactate. Due to it, the junctional flux of lactate benefits tumor growth through (1) providing an extracellular pH (pHe)-insensitive route for discharging metabolic wastes from glycolytic PDAC cells, and (2) alkalizing neighboring cells such as fibroblasts. It is suggested that blocking Cx43 channels by the specific blocker such as inoxynil and ioxynil octanoate might be a promising strategy to inhibit PDAC progression. However, more in vivo experiments using animal models are needed to test the anti-cancer efficaciousness of these specific blockers of Cx43 channels.

NHEs

In addition to the abovementioned MCT family isoforms, NHEs have been identified to participate in the regulation of intracellular pH (pHi) and pHe. Ten isoforms of NHEs have been identified (NHE1-NHE10). Of these, NHE1 has been studied most broadly. NHE1 plays a housekeeping role in the regulation of cell volume, cell proliferation, differentiation [48]. Activation of NHE1 inducing elevated pHi was reported to be an early event of oncogenic transformation, and NHE1 was proved to be essential for the maintenance of the malignant phenotype, which promotes

cancer progression [31, 32, 49-51]. Importantly, oncogenic activation of NHE1 drives both intracellular alkalization and extracellular acidification, which prompts us that NHE1 might be a key initiator for promoting malignant transformation of solid tumors. As identified to accumulate in the leading edge of migrating cells, NHE1 plays a central role in cell migration and invasion [52]. NHE1 activity is modulated by phosphorylation of seine/threonine residues and/or binding of regulatory proteins to specific sites of the cytoplasmic tail. In pancreatic cancer cell lines (BxPC and PANC-1), Ulrike Olszewski et al. has reported that NHE1 was stimulated through the up-regulation of neurotensin (NT)/neurotensin receptor 1 (NTR1) signaling pathway, leading to promote TMA, thereby favoring the invasiveness of pancreatic cancer cells at a very early stage of tumor development [53]. Furthermore, Hui Wang et al. has revealed that LAMC2, laminin subunit gamma 2, activated Akt signaling, which subsequently triggered NHE1 up-regulation, resulting in acceleration of TMA, thereby enhancing the invasiveness of pancreatic cancer cell lines (PANC-1 and AsPC-1) [31]. Interestingly, epidermal growth factor (EGF) promoted the interaction between NHE1 and EGFR via the scaffolding protein Na+/H+ exchanger regulatory factor 1 (NHEF1), driving both basal and EGF-stimulated three-dimensional growth and early invasion via invadopodial ECM digestion in PDAC cells lines (PANC-1, BXPC3, MiaPaCa-2 and CAPAN-2) [54]. Consequently, inhibiting NHE1 by its specific blockers might potently abrogate the PDAC development and progression. However, because NHE1 is best-characterized to be crucial for regulating the fluid homeostasis and secretion in the epithelial acinar and duct cells such as saliva, pancreas, small intestine, it is believed that numerous severe side-effects unwanted might occur.

Besides, other NHE isoforms, such as NHE6 and NHE9, have also been confirmed to be essential for tumor pH regulation, carcinogenesis, and development of chemoresistance [55, 56]. NHE7 localized in the trans-Golgi network (TGN) played a role in acidifying the TGN and controlling pHi in PDAC cells [57]. NHE7 was up-regulated in PDAC tumors, and correlated with poor prognosis and patient survival [58].

NBCs and CAs

In addition to H+ and lactate, the excessive extrusion of CO2 and limited distribution of blood vessels in tumor sites contributes to extracellular CO2 hydration, which results in acidification of TME. NBCs play a specific role in pHi regulation through moving HCO3- into cells and compensate for intracellular H+ [59]. There are five identified NBCs including NBCe1, NBCe2, NBCn1, NDCBE, and NBCE [59]; each of these exhibits distinct biochemical and pharmacological properties. The previous studies elucidated NBC-mediated acid extrusion mechanisms in vitro and in vivo in a variety of cancer cells, and the biological functions of NBCs on promoting cancer cell growth and progression [33, 60, 61]. Notably, NBCn1 (SLC4A7) was identified to be essential for ErbB2-mediated pHi regulation in breast cancer tissue through accelerating a pathway for cellular uptake of HCO3-. Specifically, using NBCn1-KO mice, the authors indicated that disrupting NBCn1 expression abolished ErbB2-mediated development and progression of breast cancer cells, and decelerated tumor growth [62]. Moreover, other studies indicated that NBCe1 (SLC4A4) was a vital regulator of the proliferation, migration and invasion of colon cancer cells (LS174 and DLD I) [61], breast cancer cells (MDA-MB-231) [61], and prostate cancer cells (LNCaP and PC3) [33]. In addition to NBCe1, it was demonstrated that inhibition of NDCBE (SLC4A8) and/or NBCE (SLC4A10) also weakened breast cancer cell growth [63]. However, it is obscure whether NBCs are involved in the development and progression of PDAC. More works should be addressed to investigate their role in PDAC progression in future.

Additionally, conversion of CO2 to HCO3- and H+ is catalyzed by CAs that are the transmembrane zinc metallo-enzymes. In mammals, there are five cytosolic forms (CA I, CA II, CA III, CA VII, and CA XIII), five membrane-bound enzymes (CA IV, CA IX, CA XII, CA XIV, and CA XV), two mitochondrial forms (CA VA and CA VB), and a secreted CA isozyme (CA VI) [64]. Among CA isoforms, CA IX is best characterized to be critical for regulating pHi [65] and acidifying extracellular environment [66]. CA IX is tightly associated with promotion of the aggressive/invasive phenotype of tumors [67]; importantly, it was identified to be a prominent biomarker of poor patient prognosis for many solid cancers [68]. CA IX that was activated by the HIF-1α pathways under hypoxic conditions played an important role in maintaining the hypoxic tumor microenvironment, which promoted tumor growth [68]. Structurally, CA IX consisting of an N-terminal proteoglycan-like domain, a CA domain, a transmembrane anchor that is associated with plasma membrane via a single-pass transmembrane region, and a C-terminal cytoplasmic tail, is responsible for ameliorating the CO2 hydration as well as accelerating CO2 diffusion and H+ mobility in the tumor tissue. Moreover, it was demonstrated that CA IX spatially and functionally cooperated with a variety of acid extruders and HCO3- transporters such as NBCe1-B, NBCn1 [69-71], and/or lactate and H+-exporting MCT1, MCT4, and NHE1 [72]. Recently, CA IX has recently been identified to be a pro-migratory factor facilitating cell movement and invasion by weakening intercellular adhesion and increasing cell dissociation via alleviating E-cadherin binding to β-catenin [70, 73]. In PDAC, McDonald et al. revealed an important role of CA IX in mediating the survival of pancreatic cancer cells by modulating pHi and glycolysis under hypoxic conditions [74]. Furthermore, Yuji Li et al. reported that knockdown of CA IX expression markedly inhibited the invasiveness and metastasis of PDAC cells lines (AsPC-1 and Miacapa), suggesting a pro-tumorigenic role of CA IX in initiating the PDAC progression [75]. Together, blocking the functional roles of CA IX might be an up-and-coming solution to alleviate the PDAC development and progression.

V-type H+ ATPases

In contrast to MCTs, NHEs and NBCs, V-ATPases that are ATP-driven enzymes, are abundantly present in endosomal-lysosomal system, Golgi apparatus, and other intracellular vacuoles and exclusively found in plasma membrane of specialized cell types and some cancer cells [76, 77]. V-ATPases play a cardinal role in acidifying these intracellular vacuoles, by facilitating localized concentration of H+ in acidic vesicles of the endocytic and exocytic pathways [78], which contributes to regulate important cellular processes including receptor endocytosis and vesicular trafficking [79]. Furthermore, V-ATPases provide the H+ motive force required for the formation of synaptic vesicles and subsequent accumulation of neurotransmitters [80]. In pancreatic cells, cellular pHi-dependent V-ATPase activation is crucial for insulin exocytosis [81]. In addition, V-ATPases govern the fission-fusion balance of vesicular system by interacting with Soluble NSF Attachment protein Receptor (SNARE) and GTPase [82]. Structurally, V-ATPase is a rotary nanomotor consisted of multiple subunits. Subunits are arranged in two domains: a peripheral V1 domain, which is responsible for ATP hydrolysis and an integral membrane domain V0, which is responsible for H+ translocation [83]. V1 comprises subunits A-H, V0 subunits (a, d, e, c, c'), and accessory subunit Ac45. The structure of V-ATPase is highly conserved amongst all eukaryotic cells and is involved in diverse functions throughout species.

In the context of cancer, plasma membrane V-ATPases are necessary to maintain not only an alkaline intracellular environment that is favorable for cancer cell growth, but also an acidic extracellular environment that favors cancer cell invasion [84]. Specifically, the elevated expression levels of plasma membrane V-ATPases were found in metastatic breast cancer cells [85], and blocking V-ATPases by specific inhibitors such as bafilomycin and concanamycin A (ConA) diminished the invasiveness of these cells in a manner proposed to involve plasma membrane-localized V-ATPases. Interestingly, the similar effects of blockade of V-ATPase activity were observed in melanoma cells [34] and prostate cancer cells [86, 87].

V-ATPase function is of particular interest in PDAC, given the reliance of this exceptionally aggressive cancer on nutrient scavenging, and increased lysosomal catabolism, processes critically dependent on V-ATPase activity. In PDAC patient tissues, expression levels of V-ATPase subunits V1E and V0c were correlated with cancer stage. Nonetheless, contrary to what was found in other cancer cells, V-ATPase inhibition did not consistently weaken the invasiveness of PDAC cells. Concanamycin-induced blockade of V-ATPase activity enhanced MMP2 activity, but weakened MMP9 activity [88]; however, in general, the inhibition of V-ATPase activity alleviated the invasiveness of PDAC cells [88]. Furthermore, V-ATPase activity is confirmed to be essential for degrading MT-MMP [89, 90], which was important for promoting the invasiveness of PDAC cells [91, 92]. Hayashi et al. previously reported that vacuolar V-ATPases was essential for regulating the transport of H+ from cytosol into endocytic organelles and secretory vesicles, which are responsible for transporting H+ through plasma membrane [93], indicating an important role of vacuolar V-ATPases in promoting extracellular acidification in PDAC cells. However, more studies are needed to reveal all aspects of functional roles of V-ATPases in regulating PDAC progression before establishing the drug program development that targets V-ATPases for treatment of patients with pancreatic cancer.

Conclusion

In total, I have presented the major effects of the TMA on facilitating progression of PDAC, indicating that TMA may be considered as a hallmark of PDAC. In fact, the current mechanisms underlying the TMA-mediated regulation of PDAC progression is poorly understood, and thus, more in-depth studies should be conducted to improve our knowledge of the relationship between the TMA and PDAC. This, in turn, may open a therapeutic window for pancreatic cancer treatments. In this review, the roles of H+ transporters (NHEs, NBCs, and MCTs) and pumps (V-ATPases) in facilitating the TMA by providing the optimal conditions for MMP degradation in the ECM, may play a critical role in the invasiveness of PDAC cells (**Figure 1**). Therefore, blockade of such H+ regulators by the specific blockers would be a promising approach that may provide a possible treatment for patients with pancreatic cancer.

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Ethical policy

No research involving experimentation on human or animal subjects was conducted.

Author contributions

The author contributed solely to the work.

Competing interests

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