Cancer and Metastasis Reviews (2019) 38:149–155
https://doi.org/10.1007/s10555-019-09787-4

Acidosis and cancer: from mechanism to neutralization

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Published online: 26 February 2019

Abstract
The extracellular pH of solid tumors is unequivocally acidic due to a combination of high rates of lactic acid production (a consequence of fermentative glycolytic metabolism) and poor perfusion. This has been documented by us and others in a wide variety of solid tumor models, primarily using magnetic resonance spectroscopic imaging (MRSI). This acidity contributes to tumor progression by inducing genome instability, promoting local invasion and metastases, inhibiting anti-tumor immunity, and conferring resistance to chemo- and radio-therapies. Systemic buffer therapies can neutralize tumor acidity and has been shown to inhibit local invasion and metastasis and improve immune surveillance in a variety of cancer model systems. This review will revisit the causes and consequences of acidosis by summarizing strategies used by cancer cells to adapt to acidosis, and how this acidity associated with carcinogenesis, metastasis, and immune function. Finally, this review will discuss how neutralization of acidity can be used to inhibit carcinogenesis and metastasis and improve anti-cancer immunotherapy.

Keywords Acidosis · Carcinogenesis · Metastasis · Neutralization · Immunotherapy

1 How do cancer cells adapt to acidosis?

The extracellular pH (pHe) of solid tumors is acidic, ranging from 6.5 to 6.9, whereas the pHe of normal tissues is significantly more alkaline, 7.2 to 7.4 [1, 2]. As proposed by the "acid-mediated tumor invasion" hypothesis, solid tumors export acid into the surrounding parenchyma, where this acid will induce normal cell death and promote extracellular matrix degradation *via* stimulation of release of proteases *via* increased lysosomal recycling [3]. For cancer cells to survive and thrive in this chronically acidic environment, it is axiomatic they need to evolve mechanisms of adaptation. One of these mechanisms involves chronic autophagy. Autophagy is an evolutionarily survival conserved catabolic mechanism that is used by cells exposed to stress to maintain homeostasis through self-digestion [4]. We and others have shown that many tumors upregulate autophagy under starvation and acidic conditions; our work particularly demonstrated that acid adaptation leads to chronic autophagy [5–7], leading to a therapeutic vulnerability. Indeed, it has been shown that autophagy can be targeted as a therapy for some tumor models [8]. Furthermore, not only cancer cell autophagy, but stromal cell autophagy can also be targeted. This has been shown in a pancreatic ductal adenocarcinoma (PDA) model wherein autophagic stromal-associated pancreatic stellate cells (PSCs) secreted alanine that was used by cancer cells as a nutritional source, and targeting those stromal PSC cells led to inhibition of cancer progression [9]. Another adaptation mechanism is increased lysosomal turnover and redistribution. Lysosomes are cell organelles that contain enzymes responsible for cell digestive processes; therefore, they help maintain homeostasis of cellular macromolecular components. They require acidic pH to function efficiently [10]. We and others have demonstrated that chronic acidosis increases the turnover of lysosomes and exosomes, helps the cells pump the extra protons of cytoplasm into the lysosomes using ATPase proton pumps and stabilizes the cytoplasmic pH [11–13]. Furthermore, our work demonstrated that redistribution of the lysosomal protein, LAMP-2, can be an immunohistochemical (IHC) marker for acidosis. With discovery proteomics, we observed that lysosomal proteins are profoundly upregulated following acid adaptation, and that this is accompanied by redistribution of lysosomal proteins to the plasma membrane where they inhibit acid-mediated toxicities. Specifically, we have shown that the exofacial lysosomal-associated membrane protein LAMP-

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2 is a marker for acid adaptation *in vitro* and in clinical samples, where it potently stains the peri-luminal area in DCIS [14]. Similar findings have been demonstrated by other groups in pancreatic and esophageal cancers [15, 16]. Interestingly, the lysosomal redistribution by chronic acidosis has been shown to blunt mTOR signaling by separating mTOR from Ras homolog enriched in brain (RHEB) that, in turn blocks the circadian clock [17].

Another commonly observed adaptation is a dramatic increase in adiposomes; intracellular lipid droplets (LDs) [18, 19]. LDs are organelles that function as a fat storage as well as involved in other cells processes, including, lipid metabolism, cell signaling, inflammation, and cancer [20, 21]. Notably, LDs are most intensely studied in the etiopathology of fatty liver disease and may reflect adaptation to oxidative or acidic stress [22, 23]. It has been demonstrated, using different breast cancer cell lines, that the degree of aggressiveness positively correlates with the LDs [24]. Adaptation to acidosis by increasing intracellular pH (pH_i) renders several metabolic enzymes essential for survival including GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and its paralog GAPDHS, which catalyze the sixth step (and a principal junction) of glycolysis; those observations were demonstrated recently using an *in silico* systems approach coupled with *in vitro* testing and validation [25].

Thus, adaptation of cells to chronic acidosis involves multiple pathways that are likely interrelated, although the connections are not yet well documented: autophagy, lysosome redistribution resulting in secretion of cathepsin proteases and altered mTORC activity, altered intracellular pH, and adioposomogenesis. All of these adaptations can be exploited to reveal therapeutic vulnerabilities.

2 What is the impact of acidosis?

Acidity induces metastasis. It has been observed that an acidic pHe is important, and perhaps necessary, for the transition from an *in situ* to an invasive cancer. By facilitating invasion, an acidic pH_e is also a critical factor in the formation of metastases. Using a theoretical framework based on evolutionary dynamics, Gillies and Gatenby first predicted in 2004, in *Nature Reviews*, that the peri-luminal area of ductal cancers (DCIS) should be profoundly hypoxic and acidic and that this selects for aggressive cancer cell phenotypes [26]. This has been documented by observing upregulation of glucose transporter 1 (GLUT-1) and carbonic anhydrase IX (CA-IX) immunohistochemistry (IHC) (hypoxia biomarkers) that the perluminal area of DCIS is indeed hypoxic [27, 28]. More recently, peri-luminal plasma LAMP-2 has shown that these areas are also acidic [14] .This model has been updated in *Nature Reviews* every 4 years hence [29–31]. A major component of this "acid-mediated invasion" is matrix remodeling, which is induced by increased lysosomal turnover and release of cathepsins [32], as well as direct effects on stromal cells [33].

Although previously thought to be a marker of hypoxia, it has become increasingly appreciated over the past decade that a major H^+-transporting system in cancer is the plasma membrane-associated carbonic anhydrase, CA-IX, discovered by Pastorikova (see Pastorikova, this volume). Metabolically produced HCO_3^- is dehydrated in cells by CA-II into CO_2, which exits the cell, where it is hydrated by membrane bound CA-IX (or CA-XII) into $HCO_3^- + H^+$. We, and others, have determined that CA-IX is preferentially expressed in cancers and that expression increases with stage and poor prognosis [34, 35]. Notably, the pH optimum for CA-IX is ~ 6.4, and the optimum pH for CA-XII is 7.2 [35], implying that CA-IX is active at acidic pH values and can be effective in acidifying the extracellular milieu.

Analyses of lung and breast cancers have validated that tumor cells at the invading edge have different expression patterns, compared to those in the cores [36, 37]. Specifically, the edge had more immune infiltration, higher proliferation, and less apoptosis relative to the core. Cells at the invading edge also expressed more CA-IX and less CA-XII, which are both exofacial carbonic anhydrases. This is notable, as CA-IX has a much lower pKa (< 6.5) compared to CA-XII (7.1) meaning that it is more active at low pH [38]. In addition, using window chamber models, we have convincingly shown that tumors secrete acid into their surrounding stroma and that this is necessary for local invasion [3, 39].

Tumors are genomically and functionally heterogeneous [40], and this included heterogeneity of acidity [41]. With quantitative image analytics of radiographic data ("radiomics"), we and others have shown that more heterogeneous cancers have worse outcome [42], and specifically, the texture of the tumor-stromal interface has the greatest prognostic value [43–45]. In more recent work, we have combined multiparametric magnetic resonance images (mpMRI) to generate maps of distinct "habitats"; i.e. regions with specific combinations of perfusion, cell density, and matrix [46, 47], and these are being related to outcome and gene expression patterns in patients [48–50]. In addition, using window chamber models, we have convincingly shown that tumors secrete acid into their surrounding stroma and that this is necessary for local invasion [3, 39].

Acidosis also inhibits immune surveillance of cancers, and this has been reviewed [51–53]. Both lactate and acidic pH have been shown to independently inhibit immune surveillance [54, 55]. Acidification and/or lactate induces stasis of activated human and mouse CD8+ T lymphocytes that is characterized by impairment of cytolytic activity, reduced cytokine secretion, reduced expression of IL-2Ra (CD25) and the T cell receptor, TCR, and diminished activation of STAT5/(ERK) signaling [56]. This is technically not anergy [56] because anergic cells can no longer be stimulated, whereas acid-

induced static cells can. The mechanisms of this inhibition are not known with certainty and are an area of active investigation. Recently, we showed that acidic pH blocks the activation and anti-tumor functions of T cells *in vitro via* sequestration of interferon-gamma mRNA and that this is associated with metabolic changes [55]. In this study, it was also shown that neutralization of tumor acidity *in vivo* with oral buffers increased efficacy of checkpoint inhibitors and adoptive T cell transfer [56] Fig. 1. While acidifying the extracellular pH has a modest effect on the measured intracellular pH, pHi, it is also possible that the acid signal is transduced through acid-sensing GPCRs, such as TDAG8 or OGR1 [57] or acid-stimulated ion channels, ASICs, which are expressed in T cells [58].

3 Neutralization of acidity

We have shown in earlier work that neutralization of a tumor's acidic pH through oral buffers can increase the effectiveness of weak base chemotherapeutics [59, 60]. During this work, we observed in multiple systems that chronic ingestion of *ad lib* 200 mM sodium bicarbonate increases tumor pH and rarely affects growth of primary tumors, but potently inhibited experimental or spontaneous metastases [61] (Fig. 2). We also showed that this was a buffer, and not bicarbonate effect [62, 63]. More recently, we have investigated the effects of buffer therapy on the progression of genetically modified mouse models (GEMMs), such as TRAMP Prostate, KPC pancreatic, and HER-2/neu breast cancers. Initial TRAMP studies showed that commencement of buffer therapy at 4 weeks of age prevented emergence of cancer [64]. If buffer therapy was initiated after 10 weeks (after the cancers are extracapsular), it had no effect on the primary tumor, but still completely inhibited formation of metastases [65]. Other buffers were equally effective in decreasing tumor acidity and inhibiting invasion and metastasis, including the non-volatile buffer imidazole, free base Lysine, and hydroxyl-methyl-amino-mathane, TRIS [62, 66, 67]. Neutralization was also combined with immune therapy to treat cancer. There is accumulating evidence that tumor-derived acidity also plays a role in immunosuppression [52]. Solid tumors, including melanoma, are known to be acidic [68, 69]. In mice bearing B-16 melanoma xenografts, treatment with bicarbonate synergized with the T cell checkpoint inhibitors anti-CTLA4 antibody (ipilimumab) and anti PD-1. Virtually identical results were observed in Yumm 1.1 melanoma and Panc02

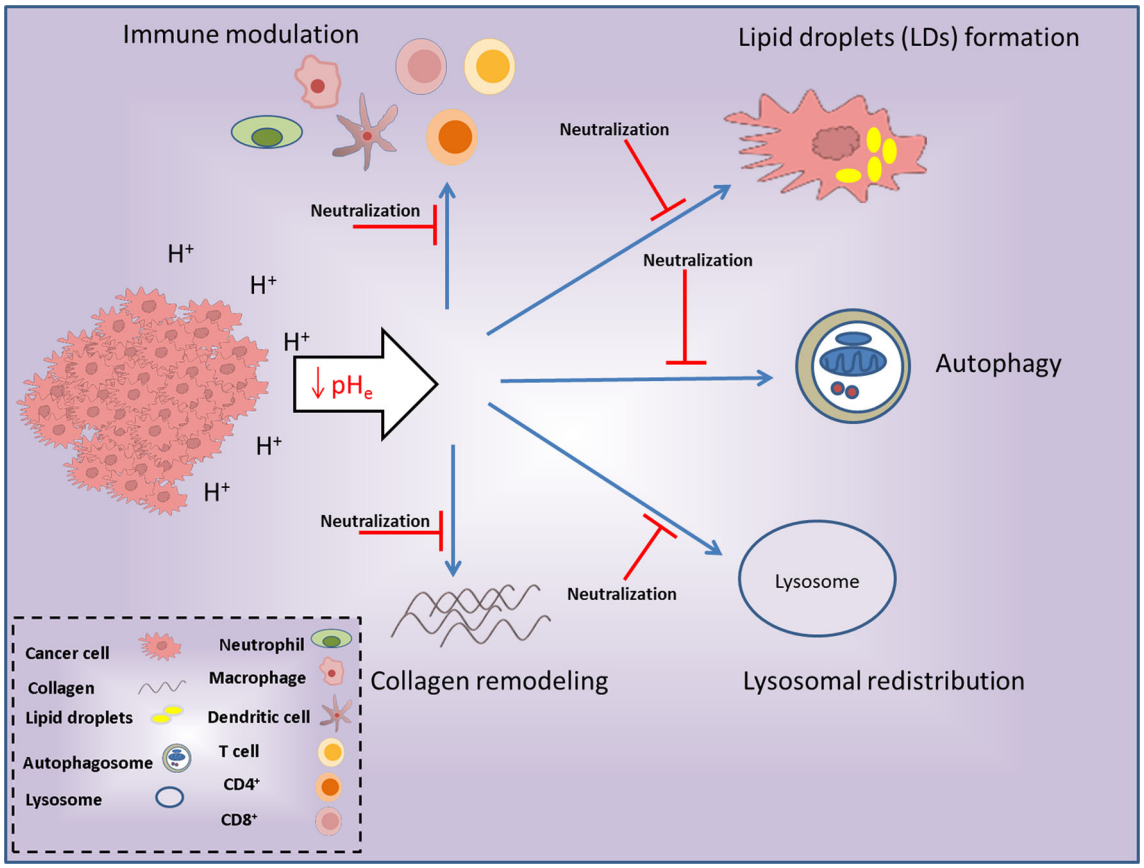

Fig. 1 Schematic diagram of tumor acidosis. Acidosis generated by tumor cells enhances adaptive mechanisms that can be inhibited by neutralization

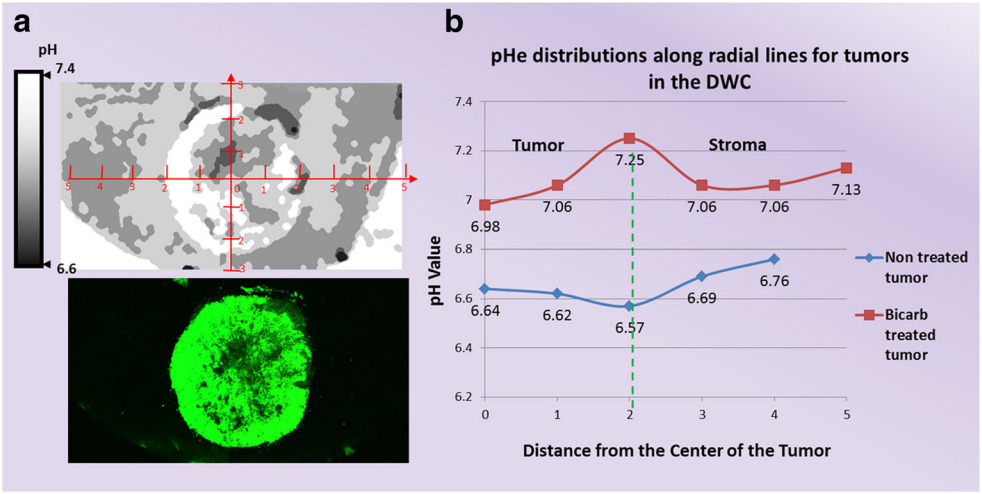

Fig. 2 The effect of NaHCO3 treatment on tumor pH. **a** Ratiometric images from SNARF-1 analysis were used to measure and compare the pH of control tumors to those tumors that were treated with 200 mmol/L of bicarbonate. The image shown here is of a bicarbonate-treated tumor. *In vitro* pH calibration was applied to the ratiometric image. pHe profiles that originated from the center of the tumor were obtained using a radial graph. pHe values were obtained along the radial lines and the edge of the tumors were defined using GFP images of the tumor. **b** pHe values corresponding to radial lines were plotted. "0" indicates the centroid of tumor and the green, dotted line indicates tumor edge

pancreatic tumor models. In adoptive cell transfer protocols, combinations with buffer therapy led to cures [70]. Notably, tumor acidity also promotes an M1- to-M2 macrophage phenotypic switch, which is pro-inflammatory, and promotes tissue remodeling and tumor progression (this has been published by others).

Despite the promise of buffer therapy, it has been difficult to translate to the clinic. Phase I/II trials for PDAC (NCT01198821) and for cancer-associated pain (NCT01846429) failed to dose escalate beyond the second dose level, primarily due to poor taste and grades 1–2 GI disturbances, leading to poor compliance [71]. As an alternative, acidosis can be directly neutralized with a CEACAM6-targeted urease, L-DOS47 (Helix Biopharma) [72]. L-DOS47 was well-tolerated and dose escalated in a phase I/II trial in non-small cell lung cancer. An alternative may also be a HCl absorbing nanoparticle, TRC101, which was shown to induce chronic compensated metabolic alkalosis in a recently completed a phase III trial in patients with chronic kidney disease (ref). The use of TC101 in treating cancer is only speculative at this stage. Additional alternatives can also be considered that will indirectly neutralize acidosis *via* targeting transport mechanisms responsible for maintaining tumor acidosis, such as carbonic anhydrase-9 (CA-IX) and monocarboxylate transporters (MCT1/4).

In conclusion, despite the extensive research in the last 10 years in acidosis and its effect on cancer, the mechanisms of adaptation to acidity, its induction of invasion and metastasis, as well as the mechanisms leading to evasion of immune surveillance are poorly understood. Furthermore, the failure of buffer therapy in the clinic emphasizes the need for alternative approaches and agents that will directly or indirectly raise tumor pH to be used in combination with chemo or immune therapy.

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