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TARGETING ACIDITY IN CANCER AND DIABETES

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Abstract

While cancer is commonly described as “a disease of the genes”, it is also a disease of metabolism. Indeed, carcinogenesis and malignancy are highly associated with metabolic re-programming, and there is clinical evidence that interrupting a cancer’s metabolic program can improve patients’ outcomes. Notably, many of the metabolic adaptations observed in cancer are similar to the same perturbations observed in diabetic patients. For example, metformin is commonly used to reduce hyperglycemia in diabetic patients, and has been demonstrated to reduce cancer incidence. Treatment with PI3K inhibitors can induce hyperinsulinemia, which can blunt therapeutic efficacy if unchecked. While commonalities between metabolism in cancer and diabetes have been extensively reviewed, here we examine a less explored and emergent convergence between diabetic and cancer metabolism: the generation of lactic acid and subsequent acidification of the surrounding microenvironment. Extracellular lactic acidosis is integral in disease manifestation and is a negative prognostic in both disease states. In tumors, this results in important sequela for cancer progression including increased invasion and metastasis, as well as inhibition of immune surveillance. In diabetes, acidosis impacts the ability of insulin to bind to its receptor, leading to peripheral resistance and an exacerbation of symptoms. Thus, acidosis may be a relevant therapeutic target, and we describe three approaches for targeting: buffers, nanomedicine, and proton pump inhibitors.

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Introduction

Acid-Base Balance

"The constancy of the internal environment (Milieu intérieur) is the condition for free and independent life" was noted by Claude Bernard in his Cahier Rouge in 1851 (translated to English in 1967[1]). While he certainly envisioned that this included pH homeostasis, it was not until 1909 that Sørensen developed the pH scale and the pH meter, and Henderson contemporaneously developed equations (later elaborated by Hasselbach) to describe the relationships between acids and conjugate bases[2]. Since that time, the measurement of pH, especially applied to biological fluids, has been a fundamental component of modern medicine. Indeed the study of acid-base balance is currently an entire field of physiology, the review of which is beyond the scope and intent of the current chapter. However, there are a few fundamental observations (Table 1) that must be accommodated within any investigation of acid-base balance. In humans, arterial and venous bloods have a pH of 7.35–7.4 and 7.20–7.35, respectively. In the steady state, catabolism is oxidative, resulting in the conversion of fats (hydrocarbons), and carbohydrates (alcohols) into carbonic or keto acids. Although body pH is strictly regulated, several diseases besides cancer and diabetes involve significant excursions of intracellular (pHi) or extracellular (pHe) pH. For example, MELAS (mitochondrial myopathy, encephalopathy with lactic acidosis and stroke-like episodes) and epilepsy are both associated with a decrease in brain cell pHi[3]. In epilepsy, correction of pH balance has been shown to ameliorate episodic seizures in some patients[4]. Musculoskeletal and cardiac function are intimately coupled to pHi homeostasis[5]. Low pH is associated with inflammation and pain (nociception) as well[6], and this is of paramount importance for both cancer and type II diabetes patients, because both inflammation and pain have a central role in the clinical manifestation of these diseases. This effect appears to have a pathogenetic mechanism for pain in the increased nociception due to the tissue acidosis[7]. As far as inflammation is concerned, a low pHe is related to the persistence of the inflammatory response and is interconnected with the sensitivity to pain[9]. Very often the symptoms may represent a central part of the disease, particularly when the disease become chronic. Primary nociceptors are acid stimulated ion channels, ASICs and hence, low pH has a key role in the transition from acute to chronic pain[8]. As we describe below, acidosis plays a major role in both cancer and diabetes, which share many pathways in common.

Measurement of intra- and extracellular pH, and its correlations with disease, is an extremely active area of research, because there is a compelling need for robust, accurate, and clinically-translatable methods of measuring pH in-vivo. Such technologies can be used as diagnostic, predictive, and/or response biomarkers, have been comprehensively reviewed elsewhere[13]. Of note, a recent study has shown that the MRI technique called chemical exchange saturation transfer, CEST, has recently been used to measure pH in a small set of human patients[9].

Metabolic Commonalities of Diabetes and Cancer.

Epidemiological evidence indicate that patients with type 2 diabetes (T2D), especially those with metabolic syndrome of dyslipidemia, hyperinsulinemia, and hyperglycemia have increased risk of developing cancers of the GI tract (liver, pancreas, colon, and rectum), as

well as breast, endometrium, and bladder[10,11]. Recent meta-analyses have indicated that the most convincing risk of T2D patients to develop a cancer is with colorectal cancer, and that, although there is a trend, the association was weaker for pancreatic, endometrial, hepatocellular and gallbladder carcinoma[12,13]. Epidemiological studies have shown that numerous risk factors are shared by diabetes and several cancer sites. Primary among these are obesity and smoking status, but also includes low physical activity and alcohol consumption. Pathophysiological mechanisms implicated in the association between T2D and cancer have been proposed for colorectal, pancreas and liver cancers. These include the T2D microenvironment, as represented by advanced glycation end-products, chronic local inflammation, hyperlipidemia, extracellular matrix disorders and altered microbiota that could predispose the development of colorectal cancer. However, despite the strong epidemiological evidence, the mechanisms of this association between diabetes and cancer are not understood.

The major sub-type of T2D is peripheral insulin resistance associated with obesity and central adiposity, leading to hyperinsulinemia and chronic inflammation, both of which have potential to exacerbate cancer risk. Hyperinsulinemia with hyperglycemia also contributes to accumulation of keto-acids, leading to chronic systemic metabolic acidosis, which is compensated by reducing HCO_3^- and reduced interstitial buffering capacity, making interstitial pH more fragile. Hyperinsulinemia is also associated with increased circulating levels of insulin-like growth factor-1 (IGF1), which is a potent mitogenic factor for neoplastic epithelial cells[16]. Binding of IGF1 to its receptor triggers activation of the PI3K → Akt → mTOR pathway, inducing metabolic activation and mitogenesis[14].

The impact of activating these pathways on acid production is exacerbated by mitochondrial dysfunction, which is commonly observed in both T2D and cancers through activation of oncogenes or inactivation of tumor suppressors[15,16]. In cancer cells, mitochondria are one of the main signaling targets for oncogenic and tumor suppressors, resulting in reprogramming of cellular metabolism. This signaling process leads to dysfunctional shapes (fragmentation/fission) of mitochondria mediated by activation/upregulation of dynamin-related protein 1 (DRP1), matching the metabolic demands of the tumor cells[17,18]. Notably, T2D-associated hyperglycemia leads to the increased production of mitochondrial ROS, causing mitochondrial fragmentation/fission via activation/upregulation of a fission protein, dynamin-related protein 1 (DRP1), and downregulation of mitofusin 2 (Mfn2). Increased fragmentation/fission of mitochondria is linked to various types of symptoms observed in T2D including insulin resistance[19]. Although further information is required to clarify the role of mitochondrial fragmentation and fission in the symptoms observed inT2D and cancer, both T2D and cancer are associated with high proton production rates due to mitochondrial dysfunction.

However the most important common feature between cancer and diabetes is the increased reliance on glucose fermentation. The continuous glucose fermentation can lead to lactate production and a significant local acidosis in both diabetic peripheral tissues and in tumors. Acidosis is exacerbated if combined with decreased perfusion, which can be a consequence of inflammation, peripheral vascular resistance, or dysangiogenesis, all common syndromes in cancer and diabetes. There is significant evidence, presented below, that this local acidosis

in cancer, can promote tissue remodeling, local invasion, metastasis, and inhibition of immune surveillance. In diabetes, local and systemic acidosis reduces insulin's affinity for its receptor, exacerbating the spiral of peripheral insulin resistance. Consequently, targeting acidosis would be an important therapeutic approach in both T2D and cancer, as discussed below.

A common pathway observed in both cancers and diabetes is the phosphatidyl inositol-4,5-bisphosphate 3-kinase, PI3K, cascade, resulting in production of inositoltris-phosphate, IP3, which is a potent activator of Ca^{2+} release from intracellular stores. Chronic activation of the inositol phosphate cascade can occur in either the PI3K catalytic subunit alpha, PIK3CA, or inactivation mutations of the phosphatase and tensin homolog, PTEN, gene, are among the most commonly observed metabolically-associated alterations in cancers[20]. Hence pharmacological inhibition of PI3K is an attractive pan-cancer therapeutic target. PI3K inhibitors have been in clinical trials, and clinical benefit has been reported in a phase I setting[21,22]. In these trials, however grade 3 or higher fasting hyperglycemia was a common (>30%) and sometimes limiting adverse event. As the PI3K enzyme is also responsible for insulin signal transduction, it was hypothesized that its inhibition would lead to blunting of the insulin signal (mimicking T2D), leading to hyperglycemia and hyperinsulinemia. This was recently investigated pre-clinically, where it was shown that the hyperinsulinemia could suppresses the effectiveness of PI3K inhibition in mouse models[23]. Further, suppressing the hyperglycemia/hyperinsulinemia response, most notably by a ketogenic diet, reduced levels of phosphorylated insulin receptor, pAkt and pS6 and could re-sensitize tumors to PI3K inhibition. This illustrates the close interplay in the metabolism of cancer and diabetes.

Tumor Acidity

Solid tumors, regardless of cancer type, are characterized as being highly heterogeneous, at the genomic, anatomic, physiologic and metabolic levels. The proximal cause of this heterogeneity is the "chaotic" and abnormal tumor vasculature, which leads to different microenvironments with different perfusion characteristics[24]. These perfusion deficits select for cells that express metabolic phenotypes that are most fit in these different environments, and these metabolic phenotypes are ultimately under control of the epigenetic and genetic landscape.

A common metabolic phenotype observed in solid tumors is elevated rates of fermentative glycolysis, i.e. the non-oxidative conversion of glucose to lactic acid. While this can be induced as an adaptive response to poor oxygenation (the "Pasteur Effect"), a remarkable century-old observation is that this glycolytic phenotype can be hardwired, and thus cancers ferment glucose, even in the presence of adequate oxygen (the "Warburg Effect"). This glycolytic switch likely occurs early in cancers, during the avascular phase of carcinoma in-situ, CIS[25], wherein cancer cells that express this phenotype are more likely to survive than ones who do not[26]. Although the mechanism and drivers of aerobic glycolysis are still debated, it is an unequivocal fact that tumors produce copious amounts of non-oxidized acids as result of elevated glucose fermentation[27]. In combination with perfusion deficits, this results in the accumulation of acids in the extracellular environment and an acidic tumor pH, with values as low as pH 6.5[28-31].

An acidic microenvironment strongly influences cancer progression[32,33]. Although it is initiated early in carcinogenesis, this phenotype is retained as cancers become locally invasive, a process known as “niche engineering”. Acidosis promotes tumor progression by stimulating invasion and metastasis [26,32,34,35], can be toxic to normal cells and mediate degradation and remodeling of the extracellular matrix [36], can elevate angiogenesis through the release of VEGF[37], and can inhibit immune surveillance by inducing T-cell stasis[38].

An acidic environment has also been shown to induce genomic instability and is an evolutionary selection force for aggressive clones of cells that are acid-adapted, leading to genomic diversity [33]. Further, the metabolic adaptations to acidity can result in large changes in the epigenome[39]. As acidity is evident in early cancers, it can be inferred that this contributes to intratumoral genetic heterogeneity[40–43], which is a proximal cause of malignance and resistance[44]. In addition to genetic heterogeneity, it has been shown in imaging studies that tumors are also physiologically and anatomically heterogeneous and that this is related to poor prognoses [45–47]. This can be quantified by converting medical images to mineable data (“radiomics”) of entire tumors[46,48–50] or definable sub-regions in tumors with specific combinations of perfusion, cell density, and extracellular matrix (ECM)[51,52]. Notably, invasive edges of tumors have habitats that are physiologically distinct from the tumor cores, and the larger the differences, the worse the prognosis [53].

At the microscopic level, invasive edges are complex mixtures of multiple cell types, including cancer cells, fibroblasts and immune cells. The tumor cells at the invading edge have distinct protein expression patterns, compared to those in the tumor cores[54,55]. Specifically, cells at the edge are more proliferative, less apoptotic, and have increased expression of GLUT-1 and CA-IX relative to the tumor cells in the core. The membrane bound exofacial CA-IX is notable as it has a much lower pKa (<6.5) compared to other carbonic anhydrases CA-XII (7.1) and thus, CA-IX is more active at low pH [56,57], and acts to acidify the tumor-stroma interface[58]. Intravital microscopy shows that invading tumors secrete acid into their surrounding stroma[36,59], which induces ECM remodeling and local invasion, driven by increased lysosomal turnover, the release of cathepsins, collagen re-organization and the release of inflammatory cytokines by stromal fibroblasts [60–64].

Another important sequelae of acid release into the stroma is immune evasion through inhibition of T-cell activation or induction of a macrophage phenotypic switch [38,65,66]. Microenvironmental acidosis reduces the effector function of tumor infiltrating lymphocytes (TIL), with reduced secretion of IL-2, up-regulation of CD25, and activation of STA5/ERK signaling[67–69]. Recently it has been shown that acidic pH blocks the activation and anti-tumor functions of T-cells via inhibition of interferon-gamma translation and that this is associated with metabolic changes[65].

Microenvironmental acidity is also involved in an increased rate of endosomal-lysosomal trafficking[64,70], and increased release of extracellular vesicles (EVs) by tumor cells [71,72]. This might be due to a need to eliminate waste, including excess acid [73]. It is thus conceivable that cancer cells under the pressure of a very harsh microenvironment need to eliminate more toxic byproducts and one mechanism at their disposal is to use extracellular elimination through exosomes or vesicles. One example is the elimination of

chemotherapeutics through exosomes[74], thus participating to chemoresistance. However, the increased release of exosomes in *in vitro* acidic condition has been recently related to the *in vivo* condition comparing plasmatic exosomes from cancer patients to both healthy donors and patients with benign tumors. It is believed that exosomes may have a key role in tumor metastasis in both setting the metastatic niche and transforming stem cells contained in target organs[75,76]

Acidosis in Diabetes.

T2D is associated with mitochondrial dysfunction, altering the distribution of acid load[15]. Mitochondrial dysfunction observed in T2D leads patients to produce large amounts of lactic acid, due to loss of function of the TCA cycle, which contributes to a lowering of the interstitial fluid pH (Fig. 1). Additionally, systemic acid load can be exacerbated by intake of high amounts of protein, measured as potential renal acid load, PRAL, and net endogenous acid production, NEAP [77,78]. In large meta-analyses, dietary acid load was strongly positively correlated with incidence of T2D, especially in women[79,80]. Further, in diabetes, due to this mitochondrial dysfunction and a lack of blood glucose, keto-acids such as beta-hydroxybutyric acid [81] are abundant and further contribute to acidosis [82]. These ketone bodies are consumed in extra-hepatic muscle and brain for ATP synthesis [83]. Thus, patients suffering from T2D with relatively normal function of hepatic mitochondria associated with no glucose availability show lowered pH in the interstitial fluid due to a large amount of ketone bodies produced from free fatty acids in addition to lactic acid.

Acidity is one of the most important therapeutic targets for diabetes mellitus [84]. In diabetes, the chronic compensated metabolic acidosis is systemic rather than regional, as seen in cancer. Compensation results in loss of HCO_3^- buffering, which makes the interstitial pH more fragile. One consequence of the low extracellular low pH observed in diabetes mellitus is that it reduces the binding affinity of insulin to its receptor, exacerbating peripheral insulin resistance [85]. Insulin resistance is one of the most essentially important symptoms observed in type 2 diabetes mellitus: patients of type 2 diabetes mellitus with insulin resistance develop hypertension, one of the most typical clinical symptoms frequently observed as cardiovascular disorders [86–91]. Insulin resistance also leads to hyper-insulinemia, and develops vascular dysfunction, hyper-activation of sympathetic nerves, and renal failure; resulting in hypertension [91–97]. Many researchers have been trying to develop various types of drugs for treatment of T2D such as sulfonylurea, biguanide, glucosidase inhibitors, thiazolidine, dipeptidyl-peptidase (PPD) IV inhibitors, and sodium/glucose co-transporter 2 (SGLT2) inhibitors based on the premise that reducing blood sugar levels can ameliorate many of the sequelae of T2D, with no significant results and needing new treatment approaches. The continuous hyperglycemia caused by the insulin-resistance-induced poor uptake of glucose into cells such as skeletal muscles, adipocytes, and hepatocytes stimulates insulin secretion from pancreatic β-cells[98–104]. Thus, continuous hyperglycemia exhausts pancreatic β-cells, resulting in development of dysfunction and damage. Indeed, reduction of blood sugar levels through, e.g. a ketogenic diet, prevents continuous hyper-secretion of insulin. As mentioned above, the fundamental prevention and treatment of T2D should be developed based on the idea of reducing insulin resistance. We hypothesize that insulin resistance is in part caused by lowered interstitial fluid pH observed in T2D[105] and lowered

interstitial (extracellular) fluid pH reduces the binding affinity of insulin to its receptor [85]. Therefore, as a fundamental prevention and treatment for T2D, we propose to develop methods to maintain the interstitial fluid pH to normal levels, which is also a goal of cancer treatments.

Targeting Acidity

Numerous groups are working to develop therapeutic approaches to inhibit or target tumor acidity. These approaches can be divided into (1) direct approaches using oral buffers, diet, or targeted agents to raise tumor pH, (2) developing agents that sequester into cells that are in an acidic microenvironment; or (3) developing or repurposing acid-activated agents, such as proton pump inhibitors, PPIs.

Direct Targeting.—The most direct approach to neutralize tumor acidity is the administration of oral buffers, such as $NaHCO_3$. In animal models oral buffers, such as sodium bicarbonate, specifically increase tumor pH without affecting systemic pH balance, and potently inhibits experimental or spontaneous metastases [106–109]. Indeed, a commercially available mix of bicarbonate and carbonate salts (i.e. BasenPulver, Pascoe Germany) was able to control melanoma growth consistent with a buffering effect at both tumor and systemic level [110]. These responses are due to buffer effects rather than to bicarbonate *per se*, as other buffers also work [111,112]. Further, effects are also manifest in genetically engineered mouse cancer models (GEMMs). For example, in the TRAMP prostate model, the initiation of buffer therapy at 4 weeks of age prevents emergence of cancer[113], but if administered after 10 weeks (after tumors are extracapsular), it has no effect on the primary tumors, but completely inhibits metastases[114,115]. As discussed below, despite the promising pre-clinical results, it is difficult to translate buffer therapy to the clinic. Recently, Another method to directly inhibit tissue acidity is through application of urease enzyme, which converts urea to 2 NH_4^+ and 1 HCO_3^-, producing a net local increase in pH. L-DOS47 is a Jack Bean urease targeted to CEACAM6 antigen[116]. An alternative agent to directly raise pH is TRC101, which is an oral non-digested nanoparticle that increases systemic buffering by absorbing HCl from the gut, leading to compensated metabolic alkalosis. This has been tested in successful clinical trials in patients with chronic kidney disease[117].

Ion Trapping.—In normal tissues, the extracellular interstitial pH, pHe, is approx. 7.3 and the intracellular cytosolic pH, pHi, is ca. 7.2, or a pH gradient of −0.1. In acidic tissues, the pHe can be as low as 6.7, and the pHi will be maintained at 7.0–7.1, or a pH gradient of +0.3–0.4. This reverse pH gradient can be exploited to trap chemotherapeutics in the cytosol of cells that are in more acidic pHe environments. Ion trapping is well-characterized theoretically and empirically, and occurs if the non-ionized form of the drug is membrane permeant and the charged form is not. Hence, weak base chemotherapeutics are excluded from more alkaline compartments, and weak acids are sequestered[118]. Table 2 provides a summary of weakly acidic, weakly basic and complex chemotherapeutics that are commonly used to treat cancers. Notably, if therapy is designed to neutralize tumor acidity, the benefits of these agents would be reversed and hence, those with increased uptake due to tumor acidity would have reduced uptake and vice-versa. Thus, raising tumor pH with buffers or

targeting would be expected to enhance efficacy of weakly basic drugs while reducing efficacy of weak acid agents [106,107].

Acid-activated Agents.—Development of agents that are only active under relatively acidic conditions is an area of active investigation. There is a tremendous interest in developing nanoparticles that are induced to release therapeutic agents in areas at low pH of tumors, and this has recently been reviewed[119,120]. Multiple chemistries can be used in these particles, such as lipid and polymeric shells made of ionizable groups that are designed to disassemble at low pH, releasing chemotherapeutic agents [121–123] .

A well-developed class of agents that are activated by low pH are the Proton Pump Inhibitors, PPIs. As discussed previously, maintenance of intracellular pH is accomplished by a series of proton exchangers, including vacuolar ATPases (V-ATPase), Na^+/H^+ exchanger (NHE), monocarboxylate transporters (MCTs) and carbonic anhydrase 9 [124]. These proton-extruding mechanisms remove metabolic acid into the extracellular matrix and are thus key to survival, especially in an acidic milieu. Thus, depriving cancer cells of the functions exploited by these exchangers should inevitably lead to cell death due to internal acidification. V-ATPases actively participate in this process in tumor cells by pumping H^+ both from the cytosol to internal vacuoles that are rapidly turned over in the face of acidity [125]. While direct inhibition of V-ATPases with specific agents, such as bafilomycin, are effectively cytotoxic *in vitro*, they have too much toxicity *in vivo* to be effectively used therapeutically, with an LD50 (mice) of ~0.45 mg/kg. An alternative approach would be to use proton pump inhibitors (PPI) that are used worldwide as very potent antacids (i.e. omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole). Importantly, these are well tolerated, even in prolonged treatments and at very high dosages, as in patients with Zollinger and Hellison syndrome and other conditions [124–127]. .

PPIs are cysteine targeting Tetracyclic Sulfonamide prodrugs that require protonation in an acidic milieu to be activated to covalently bind to free sulfhydryls. Thus, they are effective in the acid pH of the stomach, and hence the major intended target is the gastric H^+/K^+ATPase, which is abundant and has a vulnerable cysteine. In cells of extragastric tissues, acid pH is limited to lysosomes and hence, V-ATPases are also targeted, as they have vulnerable cysteines as well[128,129]. Tumor targeting may well come from the well-characterized effect of acidic extracellular environments to stimulate lysosomogenesis with increased lysosomal turnover [64,70,130,131]. . A series of preclinical investigations have shown that PPI sensitize tumor cells and tumors to the action of chemotherapeutics, even at low doses. Further, PPIs as monotherapy can exert a potent antitumor activity, and can be associated with *in vivo* modulation of tumor pH. As with buffers, PPIs have been shown to reverse tumor immune escape, through a clear increase of the immune infiltrate within the tumor mass [66]. These preclinical data represented the background for a series of clinical studies aimed at supporting the use of PPI as chemosensitizers.

In addition, pre-clinical studies have clearly shown that Carbonic Anhydrase-9, CA-IX, is critically important in maintaining an acidic pHe, and that inhibitors of this proton exchanger may be successfully used as anti-cancer agents[124]. Multiple inhibitors of CA-IX have been developed but have in common a sulfonamide to target the active site, as well as a

large size to prevent it from crossing the plasma membrane, as the active site of CA-IX (as well as CA-IV and CA-XII) is exofacial [132,133]. Moreover, recent evidence suggests that proton pump inhibitors and CA-IX inhibitors may be successfully combined in the treatment of human cancers, particularly under low pH conditions[133], thus leading us to re-think about the use of combinations between proton exchangers inhibitors[134]. As far as the involvement of CAs in diabetes there is clear evidence that CA inhibition may represent an effective prevention and treatment of obesity that we know as a common pathway of cancer and diabetes[135]. Thus, what we are learning on cancer treatment may be highly useful in the future treatment of diabetes.

Clinical Studies

Direct Targeting of Acidity.—To translate the provocative responses of animal tumors to buffers into the clinic, phase I/II clinical trials of sodium bicarbonate monotherapy were initiated, and are described in [136]. Because of widespread complaints of GI discomfort and potential effects on edema, it has been determined that $NaHCO_3$ alone is insufficient as a buffer therapy. As an alternative to buffer therapy, Helix Biopharma has recently developed a urease targeted to CEACAM-6 (L-DOS47) to raise pH by converting endogenous urea to two NH_4^+ and one HCO_3^-. This was investigated in a clinical trial (NCT02309892) in non-small cell lung cancer patients and was shown to be well-tolerated [137]. TRC101[117] has just completed a phase III pivotal trail in patients with chronic kidney disease (NCT03317444), but not yet been investigated in relation to diabetes or cancer.

PPIs in the clinic.—In 2014, Papagerakis et al. published a large retrospective metanalysis of outcomes in 596 previously untreated head and neck squamous cell carcinoma (HNSCC) patients[138]. The major findings of this study were strong univariate associations between both histamine receptor-2 antagonists (H2RAs) and proton pump inhibitors (PPIs) with treatment outcomes showing that both PPIs and H2RAs were significantly positive prognostic factors for overall survival.

Prospective clinical studies in osteosarcomas [139] and metastatic breast cancer have been published [140]. In osteosarcomas, the clinical goal was to improve the effect of neoadjuvant chemotherapy, NAC, on the tumor lesion in the resected bone with the addition of esomeprazole the two days before the combined chemotherapy. The results showed that pre-treatment with PPI increased the effectiveness of NAC in osteosarcoma patients, particularly in the chondroblastic variant. In breast cancer, patients were divided into 3 arms: one receiving a standard cisplatin and paclitaxel (C+P) chemotherapy; and 2 arms with second with C+P plus either 120 mg or 160 mg esomeprazole the days before chemotherapy. After the course of C+P was completed, patients were further divided into two arms: one continuing esomeprazole for the following year and the other discontinuing. Results showed that the 45% of the patients that continued esomeprazole after of chemotherapy were alive at the end of the study, particularly the triple negative patients, with a significant increase of both the time to progression (TTP) and the overall survival (OS). More recently, a case series study in refractory gastro-intestinal cancer have shown that the addition of PPI to chemotherapy increased the TTP in these patients[140]. Further, PPIs were shown to increase

the efficacy of standard chemotherapy and significantly improved the quality of life of treated companion animals with in either standard treatment[142] or metronomic regimens[143].

Despite this promise, the mechanism by which PPIs exert their effects remain unknown. An intriguing hypothesis might be that PPI, as the buffer therapies as well, may induce their effect through a buffering effect on the stomach [144]. This hypothesis is based on the high level of anti-acidic effect of PPI at the gastric level, but this of course will be a matter for future studies. Additionally PPIs have been shown to have off target effects, specifically to dopamine and serotonin receptors [145]. However, it has also been shown that P-type H+/K+ ATPases may be expressed by cancer cells of non-gastric origin[146,147], but also in extragastric non-cancer conditions[148]. In any event, PPIs are well-tolerated and have shown clinical benefit.

CONCLUSIONS

Diabetes and Cancer and, indeed other pathological conditions, share a phenotype of disrupted acid-base homeostasis. In both cases, the ensuing extracellular acidity has been shown to be relevant to the diseases' etiopathology. In both bases, targeting the extracellular acidity directly has been shown to ameliorate some symptoms, providing pre-clinical or clinical benefit. Despite this promise, targeting acidity and defining the mechanisms driving acidity are still nascent areas of investigation.

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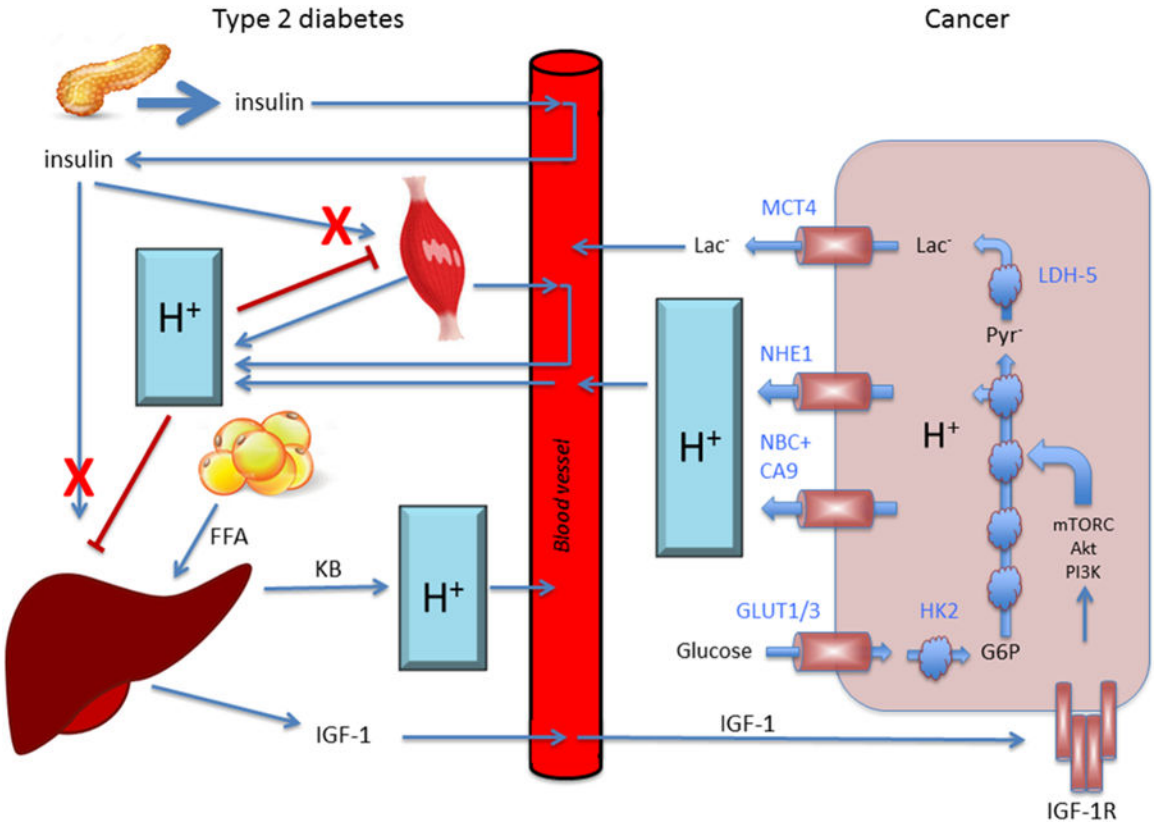

Fig. 1.

Regulation of pH and its roles in cancer and diabetic cells. The interstitial fluid pH is more highly variable compared with blood pH due to a lack of strong pH buffer. A large amount of proton and lactate^{-} are produced via metabolism in cancer and diabetic cells. A large amount of proton (H^{+}) produced systematically in diabetic cells of the whole body are circulated into other any organs in addition to cells themselves producing a large amount of proton (H^{+}). The large amount of proton (H^{+}) provides low pH environments surrounding cells in any organs including cancer and diabetic cells. These low pH environments produce insulin resistance by decreasing the affinity of insulin to its receptor, leading more proton (H^{+}) production due to diminution of glucose availability caused by insulin resistance. In the liver, the insulin resistance produces ketone bodies (KB) from free fatty acids (FFT) leading to much lower pH environments, and also releases IGF-1, which accelerates the cancer cell growth producing more proton (H^{+}).

Table 1.

Fundamental observations regarding acid-base homeostasis

1. pH is the negative log of the H^+ *activity* in solution
2. Neutrality occurs when $[H^+] = [OH^-]$ at a pH near 7.00[1].
3. All energy catabolism is eventually oxidative: i.e. the conversion of hydrocarbons and alcohols to higher oxidations states, e.g. acids.
4. Hence, the majority of regulatory systems in the body have evolved to deal with acidic pH excursions.
5. Systemic acid-base balance is achieved through the lungs, which regulate CO_2 tension, and kidneys, which regulate excretion of HCO_3^-. The aqueous CO_2 -bicarbonate system is the most important biological buffer.
6. Net diffusion of hydrogen ions in tissue occurs when bound to with mobile buffers: H^+ must be accompanied by mobile anions for charge balance.
7. Proteins provide significant non-mobile buffering power.
8. Biological processes affected by pH are primarily mediated by histidine amino acid residues, which have pKa values in the physiological range[2].
9. Multiple pathways have evolved to export metabolically-derived acids from inside of cells to their environment.

Table 2.

Common chemotherapeutic agents that are affected by the acidic pH of tumors (decreased or increased by acidic pH).

Weak bases (decreased uptake)
• Ifosfamide/Cyclophosphamide
• Erlotinib/Lapatinib/Gefitinib
• Tamoxifen
Weak Acids (increased uptake)
• Flurouricil
• Capcitabine
• Pemetrexed
Complex Ionization (decreased uptake)
• Melphelan
• Doxorubicin/Daunorubicin
• Imatinib
Complex ionization (increased uptake)
• Gemcitabine
• Irinotecan
• Sunitinib