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The Acidic Tumor Microenvironment as a Driver of Cancer

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Abstract

Acidic metabolic waste products accumulate in the tumor microenvironment because of high metabolic activity and insufficient perfusion. In tumors, the acidity of the interstitial space and the relatively well-maintained intracellular pH influence cancer and stromal cell function, their mutual interplay, and their interactions with the extracellular matrix. Tumor pH is spatially and temporally heterogeneous, and the fitness advantage of cancer cells adapted to extracellular acidity is likely particularly evident when they encounter less acidic tumor regions, for instance, during invasion. Through complex effects on genetic stability, epigenetics, cellular metabolism, proliferation, and survival, the compartmentalized pH microenvironment favors cancer development. Cellular selection exacerbates the malignant phenotype, which is further enhanced by acid-induced cell motility, extracellular matrix degradation, attenuated immune responses, and modified cellular and intercellular signaling. In this review, we discuss how the acidity of the tumor microenvironment influences each stage in cancer development, from dysplasia to full-blown metastatic disease.

COMPOSITION OF THE TUMOR MICROENVIRONMENT

The extracellular acid-base status of most normal tissue in vivo is, under physiological, well-perfused conditions, maintained relatively stable and close to that of blood (1). Accordingly, interstitial pH of normal tissue is usually in the range 7.3 to 7.4, but under some conditions, it fluctuates substantially, such as in epithelia (e.g., pancreas, colon, ventricle) during intense acid or base secretion (2) and in skeletal muscle during physical activity (3). A number of noncancer disease conditions, such as inflammatory states, ischemia, and systemic respiratory or metabolic disturbances, are also associated with interstitial acidosis (1, 4–6).

Deregulated energy metabolism, insufficient perfusion, and uncontrolled proliferation collectively cause the physicochemical composition of the tumor microenvironment to differ from that of the typical interstitium in normal tissue (7). Characteristics of the tumor microenvironment include acidity, hypoxia, increased lactate and reduced glucose concentrations, secretome changes, and recruitment of stromal and immune cells (8–13) (see **Figure 1**). Although the interstitial space of solid tumors is overall characteristically acidic (9), the composition of the extracellular tumor microenvironment is heterogeneous, ranging from areas of near-neutral extracellular pH (pH_o) to areas with intense acidification (14, 15). Thus, tumor pH_o values as low as 5.6 have been measured in human tumors, although most recorded values are in the range 6.4–7 (9, 16). It is generally reported that acidic and hypoxic regions overlap (17). However, some studies (14, 15, 18) show that areas of H^+ accumulation also occur outside of areas with O_2 depletion, for instance, in highly proliferative regions at the tumor–stroma interface. Low perfusion will typically limit both O_2 delivery and removal of acidic waste products, and elevated oxidative metabolism similarly both consumes O_2 and produces CO_2 that leads to H^+ release. In contrast, fermentative glycolysis does not require O_2 but liberates H^+. Thus, intense aerobic glycolysis can lead to regional separation of acidosis and hypoxia. The composition of the tumor microenvironment is dynamic, with blood flow and metabolism varying both spatially and temporally in the course of tumor expansion (19).

Although there is little doubt, as detailed throughout this review, that the acidic extracellular microenvironment overall promotes cancer development and progression, it is likely that pH_o in tumors can reach levels so low that even the cancer cells become restricted in their functions. Cancer cells usually have higher intracellular pH (pH_i) than normal cells when investigated under similar environmental conditions (8, 20, 21). Nonetheless, pH_i of cancer cells drops substantially—and to an almost similar extent as that of normal cells—when pH_o declines (8, 20). The frequently encountered statement that pH_i is elevated in cancer cells is therefore oversimplified. Due to the heterogeneity of the tumor microenvironment, some cancer cells will experience a very low pH_o and are consequently expected to have a relatively low pH_i (although higher than normal cells under the same conditions), whereas other cancer cells in tumor areas of relatively neutral pH_o are expected to have markedly elevated pH_i. This is likely to be especially true if the cancer cells have previously adapted to pronounced extracellular acidosis, for instance, through migration from an acidic tumor region to one of more neutral pH_o.

Appreciating the spatiotemporal complexity of the extracellular tumor microenvironment and its functional consequences is an important step toward understanding the adaptive mechanisms developed by cancer cells and identifying new therapeutic strategies targeting solid cancer tissue. Specific events and different microenvironments characterize each step in the development from normal tissue to invasive cancer. The roles of the microenvironmental acid-base conditions in shaping cancer development and progression will therefore differ between the various phases (**Figure 2**).

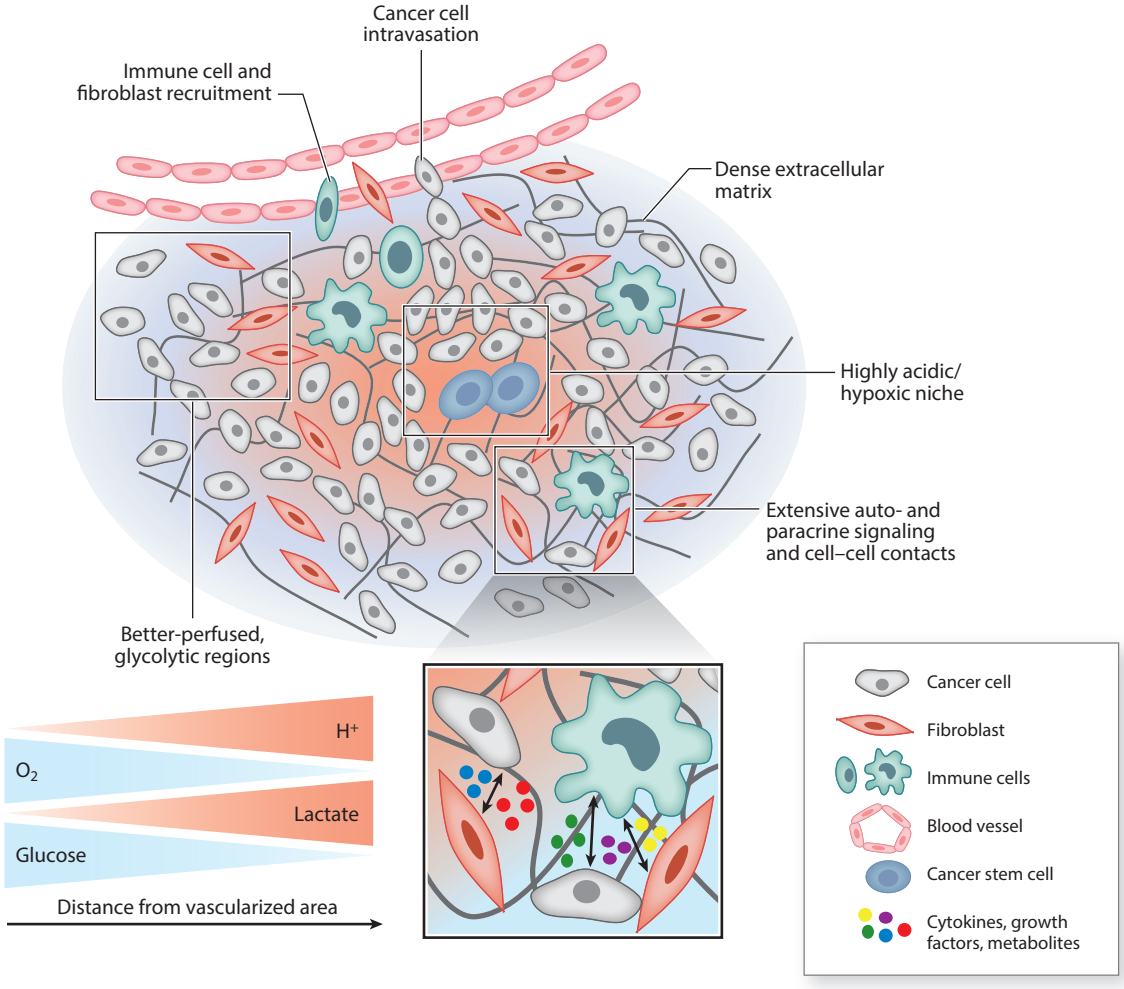


Figure 1

Schematic of a solid tumor illustrating key features of the tumor microenvironment. Reduced O_2 and glucose concentrations and correspondingly increased H^+ and lactate concentrations occur with increasing distance from vasculature. Cancer cells and multiple stromal cell types coexist in the tumor microenvironment where they interact via extensive autocrine and paracrine signals and direct cell–cell contacts. Not illustrated is the temporal heterogeneity of the microenvironment during the course of tumor development.

THE ROLE OF MICROENVIRONMENTAL ACID-BASE STATUS IN THE TRANSITION FROM NORMAL EPITHELIUM TO PRENEOPLASTIC LESIONS

Genetic and Epigenetic Changes

Genetic instability and epigenetic changes are important throughout the development of most cancers, and in particular, they are key determining factors in the transition from a normal to a preneoplastic state (22). However, even their impact at this stage is dependent on evolutionary forces represented by the microenvironment (23). Early studies show that a strongly acidic pH_o can be clastogenic and cause double-stranded DNA breaks (24). Possible underlying mechanisms

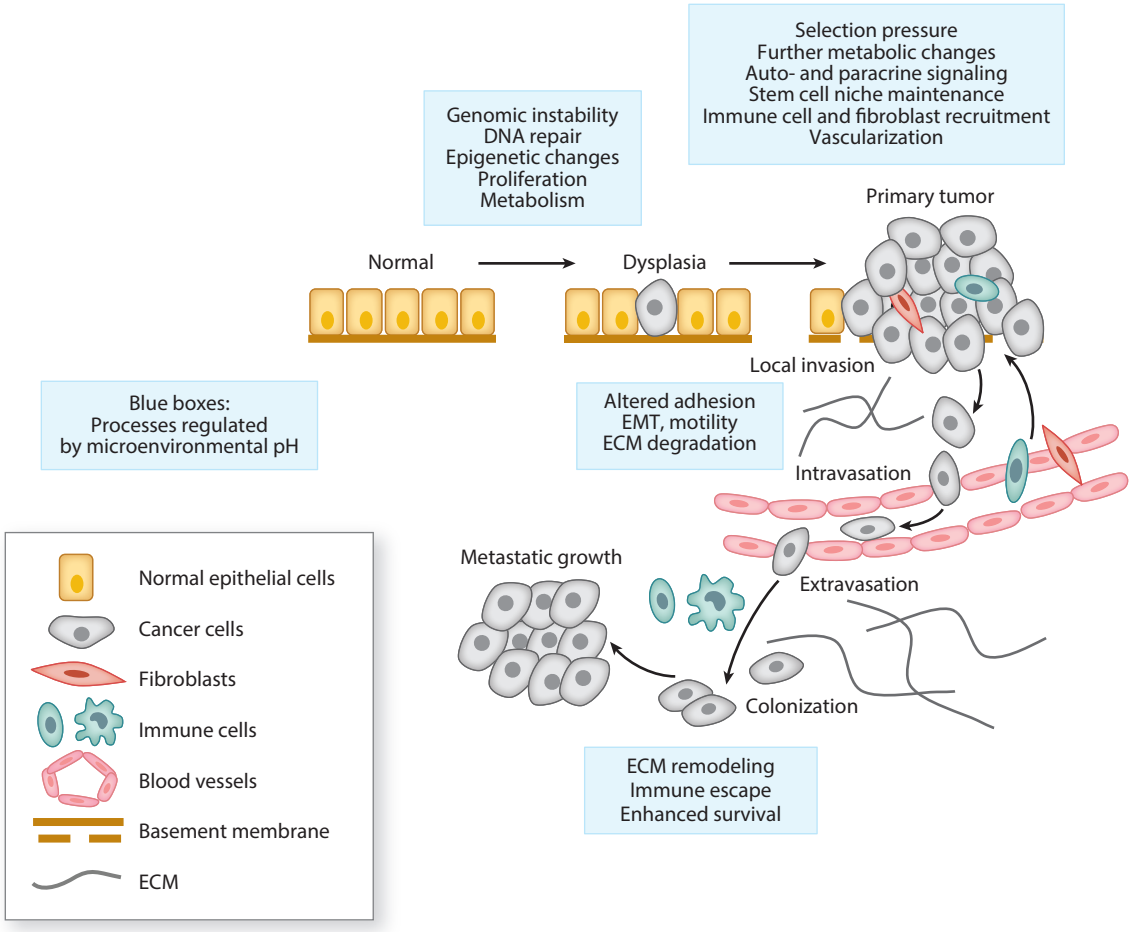


Figure 2

Overview of main processes from hyperplasia to invasiveness and consequences of the acidic tumor microenvironment. The figure shows a simplified scheme of the key processes involved in the development from a normal epithelium to a metastatic cancer. The blue boxes indicate processes with demonstrated sensitivity to extracellular pH. All of the highlighted mechanisms are discussed in the text. Abbreviations: ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition.

include acid-induced effects on topoisomerase II (25) and production of reactive oxygen species (26). Recovery from sublethal DNA damage induced by other stressors is also inhibited at acidic pH_o (with associated low pH_i), leading to accumulation of chromosomal aberrations (27). Even subtle decreases in pH_o significantly delay repair of double-stranded DNA breaks in noncancer cells (28). Taken together, acidic stress in a preneoplastic setting may increase genetic instability and hence the risk of transition to cancer. Acidic stress occurs, for instance, in inflammatory states (6), such as chronic pancreatitis, hepatitis, and inflammatory bowel disease, all of which are known risk factors for cancer (29–31). At the same time, however, an acidic environment will limit proliferation, especially of cells that have not yet acquired oncogenic mutations (see the section titled Hyperplasia and Dysplasia). These opposite effects of the acidic microenvironment, in parallel with accumulation of oncogenic mutations, may in part explain why premalignant lesions such

as pancreatic intraepithelial neoplasias (PanINs) can be dormant for decades followed by sudden, aggressive expansion (for a discussion, see 29).

Direct evidence for an adjuvant role of acidic pH_o in driving early cancer development in vivo is, to our knowledge, still lacking. Nonetheless, both cell culture work and in vivo data support the notion that acidosis can influence cancer cell epigenetics and RNA processing (14). Using the fluorescently labeled pH low insertion peptide (pHLIP), researchers recently showed that acidic tumor regions coincide with high levels of carbonic anhydrase (CA)IX, plasma membrane lysosome-associated membrane protein (LAMP)2, lactate dehydrogenase (LDH)A, and matrix metalloproteinase (MMP)9 and MMP14 in murine tumor models in vivo (14). These findings are consistent with the idea that interstitial acidification correlates with regions of high glycolytic metabolism and invasive capacity. Notably, using pHLIP labeling to isolate cells from acidic tumor regions, the authors found that acidosis was associated with marked transcriptome alterations (14). Acidosis-associated changes were dominated by altered RNA splicing patterns and enrichment for targets of RNA binding proteins with specificity for adenosine-uridine (AU)-rich motifs, such as members of the ELAVL1/human antigen R (HUR) family (14). Interestingly, the breast cancer susceptibility protein Na^+,HCO_3^--cotransporter NBCn1 (32, 33), which is upregulated at protein expression level during chronic acidosis in normal tissue and cells (34, 35) and in cancer cells (36), is a HUR target with multiple AU-rich motifs that play key roles in upregulation of NBCn1 by the p95HER2 oncogene (37). Histone deacetylase (HDAC)2 was also upregulated in the pHLIP-isolated acidic tumor boundaries, which suggests a role for acid-induced chromatin deacetylation (14), in line with previous reports of acid-induced global histone deacetylation (38). In further support of the notion that deacetylation of histones and other proteins may be a characteristic of long-term acidosis, NAD^+-dependent activation of the protein deacetylase sirtuin-1 was demonstrated in a study of cervical cancer cells exposed to chronic acidosis (39). Finally, colorectal cancer cells exposed to chronic acidosis show extensive changes in gene expression and chromatin accessibility indicative of DNA remodeling, increased xenograft tumor growth, and liver metastasis in vivo (40).

Collectively, these data indicate that exposure to an acidic microenvironment can favor genetic instability, accumulation of chromosomal aberrations, and epigenetic changes, potentially contributing to an early transition from preneoplastic to cancerous states.

Metabolic Changes

Elevated metabolic activity and changes in metabolic pathway preference are early hallmarks of cancer and provide both the energy and chemical components necessary for the high proliferative rate of cancer cells (41). Numerous studies have demonstrated that exposure to an acidic microenvironment per se is associated with major metabolic changes akin to those characteristic of many tumors (39, 42–48). While strong focus has previously been on the marked inhibitory effect of acidic pH_i on glycolysis via the pH sensitivity of the rate-limiting steps in this pathway, it has become apparent that the metabolic changes induced—or selected for—by exposure to an acidic microenvironment are far from restricted to effects on glycolysis, as discussed below.

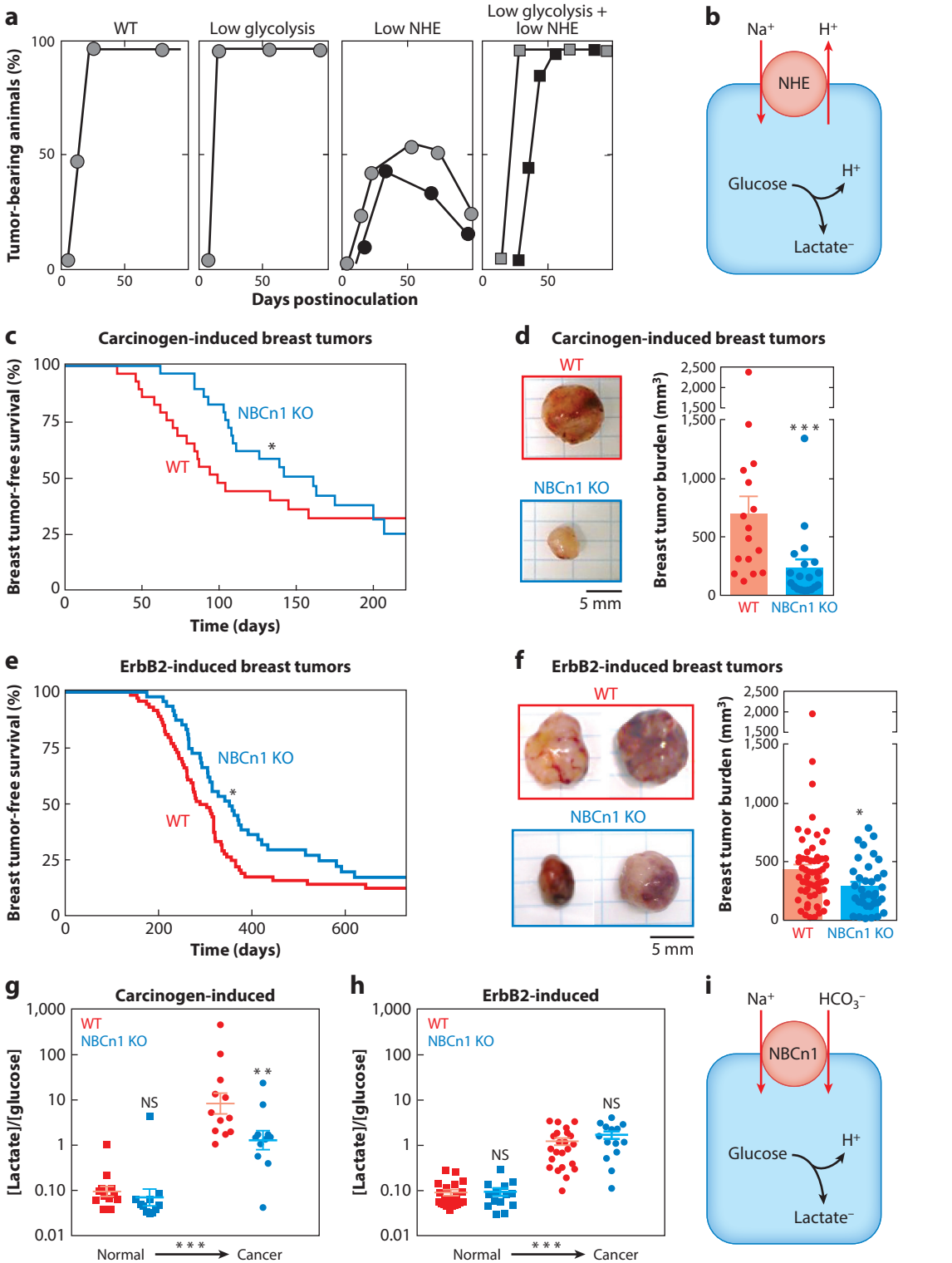
Oxidative Phosphorylation and Glycolysis

Depending on the tumor type and the microenvironmental conditions, energy production in cancer cells relies on a combination of glycolysis, oxidative phosphorylation, and other metabolic pathways (see the following sections). Metabolism contributes acidic waste products mostly in the form of CO_2 from oxidative phosphorylation and H^+ from fermentative glycolysis. Net ATP

hydrolysis adds considerably to acidification in skeletal muscle cells under intense exercise (49). However, because cancer cells in comparison show rather constant nutritional demands, relatively steady ATP levels are expected in tumors, and H^+ consumption from ATP synthesis will generally balance H^+ release from ATP hydrolysis.

The interaction between glycolytic tumor metabolism and microenvironmental pH is bidirectional, as low pH_i inhibits the enzymes involved in glycolysis, particularly the committing step catalyzed by phosphofructokinase-1 (50, 51). Thus, intracellular accumulation of H^+ from metabolism can negatively influence metabolic activity and potentially limit cancer cell proliferation and survival. Similarly, buildup of lactate can result in product inhibition of glycolytic activity. Indeed, accelerated monocarboxylate transporter (MCT)-mediated export of lactate allows tumors defective in oxidative phosphorylation to grow much faster (52). In order to avoid these inhibitory influences of intracellular acidification on metabolism and other aspects of cancer cell function, cancer cells are equipped with well-developed defense systems based on net acid extruders (e.g., Na^+,HCO_3^--cotransporters, Na^+/H^+-exchangers, and H^+-ATPases) and MCTs (e.g., MCT1 and MCT4) that remove acid equivalents and lactate from the cytosol (53, 54). As shown in **Figure 3*a*,*b***, when net acid extrusion is diminished in cancer cell lines, for instance, by interfering with Na^+/H^+-exchange activity, tumor formation is reduced in cells relying on fermentative glycolysis (55, 56). In carcinogen-induced murine breast cancer tissue with naturally high glycolytic activity, knockout of the Na^+,HCO_3^--cotransporter NBCn1—which is the dominant mechanism of net acid extrusion in both human and murine breast cancer tissue (8, 20, 32, 57)—reduces pH_i, tumor development and growth (**Figure 3*c*,*d***), and glycolysis (**Figure 3*g***), as evaluated on the basis of the interstitial [lactate] to [glucose] ratio (8). The lower glycolytic activity (**Figure 3*g***) may explain, but could also be a consequence of, the smaller tumor size (**Figure 3*d***) in the NBCn1 knockout mice (8). In tumors developing due to breast epithelial overexpression of ErbB2 (57), NBCn1 knockout still delays tumor development (**Figure 3*e***) and decelerates tumor growth (**Figure 3*f***) although the effect is of smaller magnitude and is not associated with changes in glycolysis (**Figure 3*h***). In mice with intact NBCn1 expression [compare wild type (WT) in **Figure 3*g*** to WT in **Figure 3*h***], the ErbB2-dependent tumors are, however, much less glycolytically active (57) than carcinogen-induced tumors (8) and therefore may not depend on high capacity for net acid extrusion to the same extent. This apparent correlation between relative dependency on glycolytic metabolism and benefit of inhibiting net acid extrusion (**Figure 3*c–i***) is consistent with previous xenograft studies (**Figure 3*a*,*b***) showing that the anticancer effect of abolished Na^+/H^+-exchange activity is absent in cells with low glycolytic activity due to defective phosphoglucose isomerase activity (56, 58).

Since the work of Warburg in the 1930s, it has been recognized that many cancers exhibit a characteristic metabolic shift toward glycolysis even in the presence of O_2, which has since been denoted the Warburg effect (22, 59). The advantages of this shift for tumor progression include the use of glycolytic intermediates as building blocks for growth (59) and likely also roles of lactate and acidification as oncometabolites driving multiple aspects of cancer progression (60, 61). As described above, however, acidosis, and in particular lactic acidosis, limits fermentative glycolytic metabolism. Recent work has indicated that this is not only due to the inhibitory effect on rate-limiting enzymes of the glycolytic pathway but also involves inhibition of MCT4 expression (39), stimulatory effects on mitochondrial metabolism (62–64), and shifted glucose consumption away from lactate production and toward the oxidative branch of the pentose phosphate pathway (45). As noted above, not all tumors are profoundly acidic, and substantial spatiotemporal variation in acidosis occur within tumors (14, 15), similar to what has been described for tumor oxygenation (65). Thus, the heterogeneity of tumor acidosis is in line with the well-recognized metabolic heterogeneity of tumors (66, 67) and may also support a symbiotic relationship between tumor cells



(Caption appears on following page)

Figure 3 (*Figure appears on preceding page*)

Interactions between cell metabolism, acid-base transporters, and tumor development. (*a*) Na^+/H^+-exchange is required for CCL39-derived hamster lung xenograft tumor growth but only in tumors with substantial glycolytic activity. (*b*) Schematic of a cancer cell showing the role of Na^+/H^+-exchange in eliminating H^+ released from glycolytic metabolism. (*c–f*) Knockout of Na^+,HCO_3^--cotransporter NBCn1 delays carcinogen-induced (*c*) and ErbB2-induced (*e*) breast cancer development and decelerates primary tumor growth (*d*, *f*). (*g*,*h*) Glycolytic metabolism is considerably more elevated in carcinogen-induced than ErbB2-induced murine breast cancer tissue. (*i*) Schematic of a cancer cell showing the role of Na^+,HCO_3^--cotransporter NBCn1 in eliminating H^+ released from glycolytic metabolism. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus WT under same conditions or as indicated. Abbreviations: KO, knockout; NHE, Na^+/H^+-exchanger; WT, wild type. Panel *a* adapted with permission from Reference 56. Copyright 2001, John Wiley & Sons. Panels *c*, *d*, and *g* adapted with permission from Reference 8. Copyright 2016, Springer Nature. Panels *e*, *f*, and *h* adapted with permission from Reference 57. Copyright 2018, Springer Nature.

in different microenvironmental conditions (68) (see the section titled Stromal Cell Adaptations and Cellular Interactions in the Tumor Microenvironment).

The metabolic shift in tumors toward glycolysis occurs not only in the cancer cells but also in T lymphocytes during their activation (69). If the T cells are prevented from converting glucose into lactate due to inhibition of glycolysis, important effector functions such as cytokine production are blocked (70). The reduced glycolytic activity at low pH may therefore represent one mechanism by which the acidic tumor microenvironment can contribute to immune evasion (see the section titled Immune Escape).

Glutamine and Lipid Metabolism

In addition to the impact of acidosis on the balance between oxidative phosphorylation and fermentative glycolysis, recent evidence indicates that acidosis contributes to several other metabolic changes in cancer cells. A shift toward glutamine metabolism is a characteristic feature of cancer cells (71) and has been demonstrated in cancer cells in response to acidosis lasting 24 h or more (39, 45). This is at least in part due to upregulation of HIF2α by acidosis (see also the section titled Contributions of Acidity to the Cancer Stem Cell Niche), which has been shown to increase expression of the glutamine transporter ACT2 as well as glutaminase-1 (39) that catalyzes the conversion of glutamine to glutamate.

Fatty acid metabolism is also profoundly altered in many cancers (72), and fatty acid oxidation has been found increased in acid-adapted cancer cells (42, 45). Acidosis can upregulate the sterol regulatory element-binding protein 2 (SREBP2), which is a transcription factor with key roles in control of cholesterol synthesis (46). The SREBP2-target acyl-CoA synthetase short-chain family member 2 was found upregulated in acidic cells, and its downregulation reduced growth of pancreatic cancer cells (46). In the context of lipid metabolism, it is also interesting to note that microenvironmental acidosis can induce accumulation of lipid droplets (73–75), a characteristic feature of many cancers (76).

Other Metabolic Changes

Accumulation of the α-ketoglutarate derivative 2-hydroxyglutarate (2-HG) is a metabolic hallmark of many cancer cells (41). The production of the two enantiomers *D*-2-HG and *L*-2-HG can be differentially affected during cancer development because *D*-2-HG is specifically generated by an oncogenic mutation in isocitrate dehydrogenase found in several cancers, including malignant gliomas, whereas accumulation of *L*-2-HG is stimulated by hypoxia and contributes to survival of cells under O_2-deprived conditions (48). Interestingly, acidic pH_i also increases *L*-2-HG accumulation, and this was found to be important for acid-induced HIF1α stabilization and, hence, presumably for cell survival under acidic and hypoxic stress (43, 48).

Another characteristic metabolic change in cancer cells is the opportunistic acquirement of nutrients from the environment, including macropinocytosis, increased lysosomal catabolic activity, and autophagy. In several cancer cell types, an acidic microenvironment stimulates autophagy, favoring cell survival (77–79). Interestingly, various cancers employ different metabolic strategies under acidic conditions, as illustrated, for instance, by stimulated autophagy in melanoma cells as opposed to inhibited autophagy in MCF-7 breast cancer cells upon extracellular acidification (77). Finally, acidosis can induce uptake of extracellular lipids by glioblastoma cells, resulting in accumulation of lipid droplets and increased metastatic potential (74). The major effect of low pH_o on lysosome-dependent uptake events, such as macropinocytosis and autophagy, is well in line with reports that lysosomal trafficking to the cell periphery (80, 81) and lysosomal fusion with the plasma membrane (82) increase in acidic environments.

Hyperplasia and Dysplasia

Although the precise molecular features of early-stage cancer development differ greatly between cancer types and subtypes, fundamental early steps involve dysregulation of proliferation. This results in hyperplasia, followed by early but still premalignant disruption of tissue organization in the form of dysplasia and metaplasia (22, 83).

It has been extensively documented that acidosis inhibits cell cycle progression and hence proliferation (84). Thus, cell cycle progression per se is dependent on a permissive pH_i, and a transient increase in pH_i is required for progression through specific cell cycle checkpoints (85, 86). Decreasing pH_o strongly delays cell cycle progression and growth in both naïve and acid-adapted cultured cancer cells (84, 86, 87). Notably, dual roles of acidic pH_o on proliferation are possible. Thus, while extracellular acidity will inhibit proliferation through its lowering effect on pH_i, extracellular H^+ acting directly on extracellular H^+-sensing receptors [e.g., GPR68/OGR1 (88, 89)] or H^+-activated ion channels [e.g., of the acid-sensing ion channel (ASIC) family (90)] elicits signaling events such as increases in intracellular [Ca^{2+}] that can favor proliferation.

It is expected that acidosis increases genetic instability (see above) and the propensity for mutations triggering metaplasia and dysplasia, yet the roles of pH_o in these processes have received little direct attention. It was, however, suggested that exposure to acidosis contributes to metaplasia of the cervix (83) and is part of the etiology of metaplasia in Barrett's esophagus (83, 91). Also, we previously noted that the histology of carcinogen-induced tumors differed between WT and NBCn1 knockout mice (8). Finally, elevated pH_i or overexpression of acid-extruding transporters can induce dysplasia in various models of epithelial integrity (92, 93). By extension, stimulatory roles of microenvironmental acidosis on dysplasia would likely be indirect, i.e., reflecting that acid-induced mutations or adaptations to acidosis trigger increased propensity for dysplasia once the cancer cells encounter a less acidic pH_o.

ROLE OF MICROENVIRONMENTAL pH IN THE TRANSITION FROM DYSPLASIA TO PRIMARY TUMOR

Selection of Malignant Cancer Cell Populations in the Acidic Microenvironment

The acidic tumor microenvironment, as noted above, provides a selection pressure that can give rise to cancer cell populations with more malignant phenotypes (22, 94, 95). Cancer cells are generally more likely than normal cells to gain additional mutations, and this may be enhanced by extracellular acidity (see the section titled Genetic and Epigenetic Changes). However, based on mathematical models of tumorigenesis, selection even without increased mutation rates is sufficient for evolution of tumors (96). Thus, in the absence of changes in genotype, acclimatization

to environmental stresses (e.g., hypoxia and acidosis) can alter the protein expression pattern and thus promote the acquirement of cellular abilities that, for instance, lead to treatment resistance. The cancer cells that are best equipped to survive, i.e., most evolutionarily fit, under the prevailing conditions will be selected for and expand to generate a dominant cancer cell population. Thus, it should be possible to alter the phenotype of the cancer cells and, for instance, minimize invasiveness and treatment resistance by altering the degree of acidification in the tumor microenvironment and hence the evolutionary pressure and environmental stress.

As referred to throughout this review, a wealth of recent literature has documented that when cancer cells are subjected to long-term growth (months) under acidic extracellular conditions, their genotype and phenotype fundamentally change (42, 82, 95). The phenotype, selected for by the acidic microenvironment, involves profound changes in metabolism, increased metastatic potential, and increased capacity for survival under extreme acidosis. These are all properties that potentially increase cancer cell fitness and aggressiveness, not only in the face of acidosis, but also, or perhaps especially, when reintroduced to less hostile conditions, e.g., upon invasion.

Adaptation to the tumor microenvironment is critical for the ability of cancer cells to maintain a high proliferative rate and hence for tumors to continue growing as the deranged chemical composition of the tumor interstitial space amplifies. Importantly, that does not mean that acidic pH_o per se is favorable for cancer cell growth but more likely that their adaptation to acidosis, in conjunction with oncogenic mutations, endows cancer cells with increased fitness for survival in the hostile microenvironment compared to noncancer cells. The capability of cultured human bladder carcinoma cells to proliferate under acidic conditions is dependent on CO_2/HCO_3^- (87). This is consistent with the increased importance of Na^+,HCO_3^--cotransport for maintaining pH_i in breast cancer tissue compared to normal breast tissue (8, 20, 53, 57). In principle, this ability can be acquired during carcinogenesis, for instance, if acid-base sensors on cancer cells (e.g., 88–90) induce changes in cellular expression levels of proteins involved in acid-base regulation or cell cycle progression. However, it is also possible that enhanced capacity for net acid extrusion is selected for based on naturally occurring variation observed in heterogeneous cell populations. When the Na^+,HCO_3^--cotransporter NBCn1, which is responsible for the elevated net acid extrusion capacity in breast cancer tissue compared to normal breast tissue, is genetically disrupted, breast cancer development is delayed, and cell proliferation and tumor growth rate are decelerated (8, 57, 97). However, the dependency of pH_i regulation on HCO_3^- uptake mechanisms is not universal for cancer cells as, for instance, the elevated pH_i of human colon cancer tissue relies almost exclusively on accelerated Na^+/H^+-exchange activity (21). The different transporter dependencies in various cancer forms are in line with the notion that increased capacity for net acid extrusion in cancer cells results from selection for this phenotype rather than upregulation of specific molecular mechanisms.

Contributions of Acidity to the Cancer Stem Cell Niche

Cancer stem cells (CSCs) play essential roles as drivers of tumor growth in at least some cancer types (22). The acidic and hypoxic stem cell niche is a complex and cancer type–specific environment that supports the maintenance of the CSC phenotype (98–101) (see **Figure 1**). Notably, this description is distinct from the now strongly disputed notion that acute acid stress can drive somatic cells to a pluripotent, stem cell–like state (102). While effects of microenvironmental pH on stemness have been explored for a few other cancers, they are best established for neuroepithelial tumors. In human glioma stem cells (GSCs), extracellular acidosis (pH 6.5, 6–10 days) favors expression of the stem cell markers Olig2, Oct4, and Nanog as well as angiogenic markers and HIF2α (99). Extracellular acidosis (most potently around pH 6.8) also synergizes with hypoxia to

upregulate HIF1α and HIF2α in a heat-shock protein (HSP90)-dependent manner (100). Expression of HSP90 increases during acidosis and further correlates with the stem cell phenotype (100). A subsequent study indicates that extracellular acidosis (pH 6.8) enhances the self-renewal capacity, neurosphere growth, and mitochondrial respiration of stem cell–like glioma cell cultures and is associated with upregulation of stem cell markers such as nestin, Oct4, Sox2, CD133, and the putative breast cancer oncogene CYP24A1 (101). The mechanisms of pH-regulated stemness are likely multifactorial and could include both upstream sensors of acidosis—for instance, ASIC1 and ASIC3 that are expressed in GSCs (103)—as well as downstream processes, such as metabolism and proliferation (see above), known to impact stemness. In congruence with the notion that acidic pH contributes to maintenance of stemness, a transient increase in Na^+/H^+-exchange activity, which elevates pH_i and inhibits Hedgehog signaling, is required for differentiation of *Drosophila melanogaster* adult follicle stem cells and mouse embryonic stem cells (104).

Stromal Cell Adaptations and Cellular Interactions in the Tumor Microenvironment

Cancer cells in solid tumors are embedded in a stroma of connective tissue. In addition to the blood vessels and immune cells considered below, this stroma contains a large number of cancer-associated fibroblasts (CAFs) that not only shape the tumor architecture but also contribute to the tumor-protective niche that promotes cancer progression and treatment resistance (105).

Interactions between cancer cells and stromal cells are numerous and bidirectional (106) (see **Figure 1**). The acidic tumor microenvironment—generated based on metabolic waste products, such as CO_2 and H^+, exported from the cancer cells—represents one mechanism by which cancer cells modify the composition and function of the stroma. The stromal cells have also recently been proposed to play a significant but complex role in modifying the acid-base status of the tumor microenvironment: CAFs can facilitate extracellular acidification by secreting CAIX into the tumor microenvironment (107) but may also serve as a sink for accumulated extracellular acid and, in this manner, buffer sudden pH changes (108). The possible contribution of secreted CAIX to extracellular acid-base regulation in solid tumors (107) adds to the evidence for accelerated CO_2 hydration and acidification in the extracellular space of tumors that express CAIX on the surface of cancer cells (109). Studies in 2-dimensional culture show that myofibroblasts express high levels of anion exchangers that import acid and connexin proteins that allow intercellular diffusion (108). In combination with work on 3-dimensional cultured cancer cell growths (i.e., spheroids) coupled through intercellular gap junctions, these studies suggest that cellular syncytia in the tumor space might act as alternative pathways for acid-base equivalents (e.g., protonated mobile buffers or HCO_3^-) moving between severely acidified and better vascularized tumor regions (108, 110). To what extent stromal cells contribute to setting a cancer-promoting pH_o level in malignant tumor tissue—e.g., by determining whether the transfer of acid toward well-perfused tumor regions occurs predominantly via intracellular or extracellular routes—needs further investigation in experimental preparations that more accurately resemble a realistic tumor geometry and cellular composition. Recent studies (108, 110), however, present the interesting possibility that cellular base extrusion mechanisms (e.g., AE2) and gap junctions, in addition to net acid extruders (e.g., Na^+,HCO_3^--cotransporters, Na^+/H^+-exchangers, H^+-ATPases and MCTs), are potential therapeutic targets for modifying the acid-base composition of the tumor microenvironment.

Cancer cells and stromal cells can also cooperate on the disposal of lactate. It has been proposed that lactate travels in part via an intracellular route through gap junctions (111) but is also exported by glycolytic cancer cells (e.g., via MCT4) and taken up by stromal cells or oxidative cancer cells (e.g., via MCT1) that metabolize the lactate via the citric acid cycle (112). The cellular export

and import of lactate via MCTs take place in cotransport with H^+, and thus directly eliminate the associated acid load. The MCT-assisted metabolic shuttle therefore avoids accumulation of lactate and H^+ in both the intracellular and interstitial space and allows the glycolytic cancer cells to be continuously fueled by fermentative glycolysis while providing an attractive metabolic substrate to neighboring oxidative cells. In contrast, the proposed intercellular transport of lactate via gap junctions (111) is less attractive from the point of acid-base homeostasis as it is not directly coupled to H^+ elimination and would require additional export mechanisms for acid equivalents in order to avoid massive intracellular acidification of the glycolytic cancer cells.

Cancer cells as well as CAFs, macrophages, and various lymphocytes secrete cytokines that can accumulate at high concentration in the tumor microenvironment and mediate communication between the various cell types in the tumor (13, 113) (see **Figure 1**). The tumor-derived cytokines, such as transforming growth factor β and tumor necrosis factor α, are also capable of modifying the protein expression pattern, metabolism, and acid-base regulatory function of cancer cells and fibroblasts (108, 114–116). Additionally, direct cell–cell contacts between cancer cells and stromal cells (117, 118) may play a role, for instance, in metastasis and immune cell infiltration.

Vascularization

During primary tumor expansion, and again during growth of metastases, diffusion of nutrients, O_2, and metabolic waste products between the blood supply and the cancer cells becomes inefficient as the distance to the nearest blood vessels increases (119) (see **Figures 1** and **2**). The combination of elevated metabolic activity in cancer cells and the slow rate of diffusion leads to gradual O_2 depletion and interstitial accumulation of acidic metabolic waste products that characterize the microenvironment of solid cancer tissue. The formation of new blood vessels (i.e., angiogenesis) and incorporation of existing blood vessels from surrounding tissue (i.e., co-option) partially counteract the diffusion limitations (120). As discussed in greater detail below, the tumor blood vessels may also undergo functional adaptations that promote blood flow delivery to the cancer tissue (121, 122). In fact, blood flow delivery to some tumor regions can be substantially higher than to corresponding normal tissue (123), although O_2 delivery usually remains insufficient to match the elevated metabolic demand. Notably, the level of tissue oxygenation within the tumor is not a straightforward function of perfusion because blood pO_2 values can vary substantially and be low even in some well-perfused tumor vessels (15).

Because the acidic and hypoxic tumor microenvironment facilitates not only malignant development and progression but also treatment resistance (124), the mechanisms that alter tumor vascularization or interfere with tumor vascular function are promising therapeutic targets. Notably, however, antiangiogenic therapeutics have so far had limited success. This is most likely because (*a*) angiogenesis is initiated relatively early in tumor development before the malignant process is diagnosed and treatment can commence, and (*b*) vessel co-option that is not targeted by current antiangiogenic therapy is common in primary tumors and metastases (125). It is, however, also possible that increased acidity in less vascularized tumor tissue selects for more malignant cancer cells.

It is well established that hypoxia and accumulation of growth factors promote development of new tumor blood vessels (126), whereas the signaling role of other elements of the tumor microenvironment, such as acid-base deviations, has not yet been comprehensively defined. Based on cultured endothelial cells in vitro, endothelial cell apoptosis, cell migration and proliferation, as well as expression of and signaling from vascular endothelial growth factor (VEGF) receptors are inhibited when pH_o decreases to extremely low levels of 6.4 to 6.5 (127, 128). On the other hand, transcriptional activity of VEGF has been found independently elevated by hypoxia and acidosis

in brain tumors in vivo (18); and in human umbilical cord endothelial cells, combined lactic acidosis and hypoxia increase HIF1α and VEGF levels more than hypoxia alone (129). Lactate can also lead to transcriptional upregulation of VEGF receptors and thus trigger angiogenesis independently of hypoxia (112). This complex pattern of regulation is recapitulated in the in vitro rat aortic ring model where spontaneous microvascular outgrowth is inhibited under acidic conditions, but growth factor–induced outgrowth is more pronounced at low pH compared to normal pH (130).

Intratumoral blood vessels are structurally immature compared to normal blood vessels and share characteristics of arterioles, capillaries, and venules (131). The consequent heterogeneous resistance and blood flow patterns in the tumor vasculature combined with increased permeability of the abnormal tumor blood vessels contribute to the high interstitial pressures observed in malignant cancer tissue (132). Lymph vessels in the tumor tissue also influence the degree of interstitial fluid accumulation (i.e., edema) and moreover serve as conduits for immune cells and cancer cells (133). In the context of the acidic tumor microenvironment, it is noteworthy that local interstitial pH influences the contractile function of lymph vessels: During acidosis (e.g., pH_o 6.8), rhythmic contractile responses—at least of human thoracic ducts and bull mesenteric lymphatics—markedly decline (134, 135). Lower pumping activity of tumor lymph vessels during acidosis may modify both immune surveillance of tumors and tumor metastatic propensity.

Functional Adaptations of Tumor Feed Arteries

The blood vessels that supply cancer tissue reach relatively small dimensions (diameters of ~100 μm) before they penetrate into the tumor tissue. As a consequence, extratumoral blood vessels contribute substantially to tumor vascular resistance, and changes in the contractile state of tumor feed arteries are expected to significantly modify tumor blood flow. Murine breast cancer feed arteries and human colon cancer feed arteries show functional specialization that facilitates vasodilation or inhibits vasocontraction (121, 122) and thereby minimizes vascular resistance compared to corresponding normal arteries. The mechanisms explaining the reduced vascular resistance of cancer feed arteries depend on the source of the arteries. In human colon cancer feed arteries, enhanced endothelial nitric oxide (NO) production increases vasorelaxation, which is consistent with their higher expression of endothelial NO synthase (122). Mouse breast cancer feed arteries, on the other hand, show thinner media thickness and lower α_{1A}-adrenoceptor expression, which limits vasocontraction (121). Irrespectively, lower vascular resistance will promote tumor blood flow. The functional specialization of tumor feed arteries also suggests that these arteries could be targeted in order to increase chemotherapy delivery and improve oxygenation during radiotherapy, or alternatively with the aim of metabolically starving the cancer cells by limiting their O_2 and nutrient supply.

The underlying signaling mechanisms responsible for changes in extratumoral blood vessel function are not yet clear. Tumor-adjacent blood vessels may be affected by the tumor microenvironment as the abnormal chemical composition extends several millimeters into the surrounding tissue (136, 137), but it is also possible that decreases in downstream resistance affect the tumor feed arteries as they experience sustained elevations in flow and shear stress (138).

Immune Escape

In order for primary tumors to evolve and for cancer cells to invade and establish secondary tumors—directly or through blood or lymphatic vessels—they must be able to avoid immune cell detection and/or immune-mediated cell death (22). In congruence, the degree of immune cell

infiltration is an independent prognostic predictor in colorectal carcinomas (139). The available evidence strongly indicates that the acidic tumor microenvironment inhibits antitumor immune responses, but the difficulty in experimentally modifying the acid-base conditions of the tumor microenvironment in vivo has hampered the collection of direct evidence.

Buffer therapy, where small H^+ buffers (e.g., HCO_3^- or Tris-base) are provided orally in order to neutralize the extracellular tumor microenvironment (137, 140), has shown promise as a maneuver that enhances immune-mediated responses against xenograft tumors (141). Inhibitors of H^+-ATPases have also been found to alkalinize the extracellular space of tumors and enhance tumor-targeted immune responses (142).

Previous studies show that low pH_o in tumors inhibits anticancer immune effectors (e.g., M1 macrophages, T lymphocytes, dendritic cells, and natural killer cells) and boosts immunosuppressive components (e.g., M2 macrophages and regulatory T lymphocytes; see also 10). T cells exposed to acidosis display higher activation thresholds requiring costimulatory signals (e.g., CD28 agonism) for full activation and exhibit enhanced negative regulatory signals via upregulated IFN-γ-R2 and CTLA-4 (143). Acidic extracellular conditions (pH_o of 6.5) have also been found to reduce expression of T cell receptor components (142). In contrast, the inhibitory effect of the acidic tumor microenvironment on dendritic cells is not due to H^+ that in fact stimulates antigen presentation (144). Instead, it may be explained by accumulated lactic acid, which modulates the dendritic cell phenotype and causes increased production of anti-inflammatory (e.g., IL-10) and reduced production of proinflammatory (e.g., IL-12) cytokines (145, 146). Natural killer cells are also sensitive to low pH_o and lactic acid that together reduce their tumor infiltration and activity (147, 148).

On top of acid-induced immunosuppression, hypoxia adds to the negative influence of the tumor microenvironment on antitumor immune responses. As such, targeted reduction of tumor hypoxia has been shown to restore T cell infiltration in murine prostate cancer tissue particularly when combined with checkpoint inhibitor treatment (149).

ROLES OF THE ACIDIC MICROENVIRONMENT IN SHAPING PROGRESSION FROM PRIMARY TUMOR TO METASTASIS

Long-term adaptation to acidic pH_o favors invasion in a wide range of cell types (95, 150, 151). Not all of this effect can be ascribed to evolutionary selection for invasive phenotypes, as exposure to low pH_o for as short as 24 h stimulates invasiveness of melanoma cells (150) and colorectal cancer cells (90), the latter at least in part via upregulation of ASIC2. Notably, in some cases invasion is only stimulated by acidic pH_o adaptation when cells are subsequently reacclimated to normal pH_o (95). This is again in line with the notion that the fitness advantage of acidic pH_o adaptation drives cancer progression when the cells subsequently encounter less-acidic environments.

As depicted in **Figure 2**, the metastatic cascade, during which cancer cells disseminate from the primary tumor and colonize other organs, comprises multiple, extensively studied steps. However, relatively few studies have investigated how microenvironmental acidosis influences the specific steps in the metastatic process. Most evidence is available for epithelial-to-mesenchymal transition (EMT) and extracellular matrix (ECM) remodeling, as described in the following sections.

Epithelial-to-Mesenchymal Transition and Dissemination

Several reports suggest that environmental acidosis stimulates EMT, during which epithelial cells lose their polarity and cell–cell adhesions and become mesenchymal like with enhanced migratory and invasive properties. This was shown, for instance, in pancreatic cancer cells,

where acid-induced stimulation of ASICs increased intracellular [Ca^{2+}], leading to activation of RhoA and EMT (152). Melanoma cells subjected to extracellular acidosis for 24 h also exhibited increased expression of EMT markers and augmented in vitro invasiveness, yet they did not show increased metastasis formation in vivo but rather a partial G1 arrest (153). Interestingly, coculture of acid-adapted and nonacid-adapted melanoma cells resulted in increased in vivo invasiveness, suggesting stimulatory cell–cell interactions (153), possibly involving acidosis-induced exosome release, as recently demonstrated (154).

Cell-cell adhesion, the loss of which is necessary for dissemination of tumor cells early in the metastatic cascade, has been found reduced by acidic pH_o (155). In particular, gap junctions formed between cancer cells modify whether cancer cells invade collectively or as single cells (156). This type of intercellular communication is highly sensitive to acid-base conditions, although there is considerable variation in the pH-dependency pattern between different connexin isoforms (157–159). Cell-matrix adhesion is also highly sensitive to pH_o, reflecting the pH sensitivity of integrin-ECM affinity (12).

Taken together, evidence from several cancer types indicates that the acidic microenvironment favors EMT and dissemination of cancer cells, which are prerequisites for invasive behavior.

Extracellular Matrix Degradation and Remodeling

In order to infiltrate the surrounding tissue or spread through the blood stream or lymphatic vessels, the cancer cells must first penetrate through the ECM. There is considerable evidence that the acidic pH_o of the tumor microenvironment aids this process. MMPs and cathepsins secreted from the cancer cells undergo acid-induced activation in the extracellular space before they catalyze degradation of the ECM (12). Accordingly, pericellular acidity, e.g., resulting from NHE1 activity in invadopodia, facilitates degradation of the extracellular matrix (160), and numerous studies document the important role of net acid–extruding transporters for cancer cell invasion (12, 155, 160–164). Interestingly, the dependency of metastasis on local acidification appears to vary between cancer cells and matches the level of glycolytic metabolism and hence the ability of the individual cell lines to produce and export large quantities of acid (165). Because degradation of the ECM during cancer cell invasion occurs at the outer surface of the migrating cancer cells, it is the pH in this diffusion-restricted compartment rather than the bulk pH of the extracellular space that is of particular interest. Indeed, cellular net acid extrusion, in particular via Na^+/H^+-exchange but for some cell types also via Na^+,HCO_3^--cotransport, can establish pH_o gradients that facilitate directional migration and contribute to acid-base-induced changes in ECM degradation as well as cell-matrix interactions (166–170).

CONCLUSIONS AND PERSPECTIVES

A wealth of literature demonstrates that tumors exhibit regions of marked extracellular acidity, which develop because of deregulated energy metabolism of cancer cells in conjunction with uncontrolled proliferation and insufficient perfusion. Whereas cancer cells, especially after adaptation to or selection in conditions of chronic acidosis, likely cope better with the hostile tumor environment than normal cells, highly acidic tumor regions are not conducive to growth, even of cancer cells. Because the tumor microenvironment is spatially and temporally heterogeneous, cancer cells adapted to acidosis are, however, likely to encounter more neutral regions. Here, the enhanced acid extrusion capacity and other properties endowed by the cancer cells during acidic selection will render them highly fit in evolutionary terms, with increased propensity for proliferation, survival, and invasiveness.

Interactions between cancer cells and between cancer and stromal cells—whether in the form of released metabolites, secreted cytokines, or direct cell–cell contacts—play important roles during cancer progression. During the metastatic cascade, the acidic tumor microenvironment can also facilitate EMT, ECM degradation, and cancer cell migration. The low pH_o also plays a potentially critical role by inhibiting infiltration with and function of immune cells, which may protect the growing tumor against immune-mediated destruction. Considering the complexity of the tumor microenvironment in terms of cell–cell interactions and the diffusion hindrances of the tortuous extracellular space, there is a persistent requirement for studies of acid-base regulation and their consequences in experimental preparations that mimic the geometry and structural composition of solid cancer tissue.

The cancer cell mechanisms responsible for establishing the composition of the tumor microenvironment are interesting therapeutic targets. Likewise, treatment strategies acting on acid-sensitive structural or functional interactions between cancer cells, stromal cells, and ECM provide new therapeutic options and limit the risk of treatment resistance. Due to the radical differences between the composition of the tumor and normal interstitium, treatment strategies targeting proteins that take part in establishing or sensing the tumor microenvironment are likely to have dramatically different consequences for cancer cells than normal cells in other organs. Hence, limited adverse effects can likely be achieved even for targets that are also expressed in nonmalignant tissue.

DISCLOSURE STATEMENT

Ebbe Boedtkjer is an inventor on patent applications that describe new technologies for the inhibition of acid-base transport in cancer tissue. Stine F. Pedersen declares no conflicts of interest.

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LITERATURE CITED

1. Palmer AS, Miller AJ, Davis C, Greene R. 1998. Gas tensions in cardiac lymph as a reflection of the interstitial space of the heart. *Angiology* 49:735–41
2. Niv Y, Fraser GM. 2002. The alkaline tide phenomenon. *J. Clin. Gastroenterol.* 35:5–8
3. Street D, Bangsbo J, Juel C. 2001. Interstitial pH in human skeletal muscle during and after dynamic graded exercise. *J. Physiol.* 537:993–98
4. Hosseinpour M, Khamechian T, Shahrokh S. 2014. Peritoneal potassium and pH measurement in early diagnosis of acute mesenteric ischemia in rats. *Arch. Trauma Res.* 3:e20957
5. Mochizuki S, Taniguchi M, Seki S, Ishiki M, Ozeki T, et al. 1988. Acid-base changes in ischemic myocardium and intervention with hypothermia or bicarbonate. *Jpn. Circ. J.* 52:638–45
6. Punnia-Moorthy A. 1987. Evaluation of pH changes in inflammation of the subcutaneous air pouch lining in the rat, induced by carrageenan, dextran and *Staphylococcus aureus*. *J. Oral Pathol. Med.* 16:36–44

7. Boedtkjer E, Bunch L, Pedersen SF. 2012. Physiology, pharmacology and pathophysiology of the pH regulatory transport proteins NHE1 and NBCn1: similarities, differences and implications for cancer therapy. *Curr. Pharm. Des.* 18:1345–71
8. Lee S, Axelsen TV, Andersen AP, Vahl P, Pedersen SF, Boedtkjer E. 2016. Disrupting Na^+,HCO_3^--cotransporter NBCn1 (Slc4a7) delays murine breast cancer development. *Oncogene* 35:2112–22
9. Vaupel P, Kallinowski F, Okunieff P. 1989. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. *Cancer Res.* 49:6449–65
10. Huber V, Camisaschi C, Berzi A, Ferro S, Lugini L, et al. 2017. Cancer acidity: an ultimate frontier of tumor immune escape and a novel target of immunomodulation. *Semin. Cancer Biol.* 43:74–89
11. Bahrami A, Hassanian SM, Khazaei M, Hasanzadeh M, Shahidsales S, et al. 2018. The therapeutic potential of targeting tumor microenvironment in breast cancer: rational strategies and recent progress. *J. Cell. Biochem.* 119:111–22
12. Stock C, Pedersen SF. 2017. Roles of pH and the Na^+/H^+ exchanger NHE1 in cancer: from cell biology and animal models to an emerging translational perspective? *Semin. Cancer Biol.* 43:5–16
13. Andersen AP, Moreira JM, Pedersen SF. 2014. Interactions of ion transporters and channels with cancer cell metabolism and the tumour microenvironment. *Philos. Trans. R. Soc. B* 369:20130098
14. Rohani N, Hao L, Alexis MS, Joughin BA, Krismer K, et al. 2019. Acidification of tumor at stromal boundaries drives transcriptome alterations associated with aggressive phenotypes. *Cancer Res.* 79:1952–66
15. Helmlinger G, Yuan F, Dellian M, Jain RK. 1997. Interstitial pH and pO_2 gradients in solid tumors *in vivo*: high-resolution measurements reveal a lack of correlation. *Nat. Med.* 3:177–82
16. Griffiths JR. 1991. Are cancer cells acidic? *Br. J. Cancer* 64:425–27
17. Chiche J, Brahimi-Horn MC, Pouyssegur J. 2010. Tumour hypoxia induces a metabolic shift causing acidosis: a common feature in cancer. *J. Cell. Mol. Med.* 14:771–94
18. Fukumura D, Xu L, Chen Y, Gohongi T, Seed B, Jain RK. 2001. Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. *Cancer Res.* 61:6020–24
19. Gillies RJ, Brown JS, Anderson ARA, Gatenby RA. 2018. Eco-evolutionary causes and consequences of temporal changes in intratumoural blood flow. *Nat. Rev. Cancer* 18:576–85
20. Lee S, Mele M, Vahl P, Christiansen PM, Jensen VED, Boedtkjer E. 2015. Na^+,HCO_3^--cotransport is functionally upregulated during human breast carcinogenesis and required for the inverted pH gradient across the plasma membrane. *Pflügers Arch.* 467:367–77
21. Voss NCS, Kold-Petersen H, Henningsen MB, Homilius C, Boedtkjer E. 2019. Upregulated Na^+/H^+-exchange protects human colon cancer tissue against intracellular acidification. *BioMed. Res. Int.* 2019:3702783
22. Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: the next generation. *Cell* 144:646–74
23. Gatenby RA, Brown J. 2017. Mutations, evolution and the central role of a self-defined fitness function in the initiation and progression of cancer. *Biochim. Biophys. Acta Rev. Cancer* 1867:162–66
24. Morita T, Nagaki T, Fukuda I, Okumura K. 1992. Clastogenicity of low pH to various cultured mammalian cells. *Mutat. Res.* 268:297–305
25. Xiao H, Li TK, Yang JM, Liu LF. 2003. Acidic pH induces topoisomerase II-mediated DNA damage. *PNAS* 100:5205–10
26. Zhang HY, Hormi-Carver K, Zhang X, Spechler SJ, Souza RF. 2009. In benign Barrett's epithelial cells, acid exposure generates reactive oxygen species that cause DNA double-strand breaks. *Cancer Res.* 69:9083–89
27. Jayanth VR, Bayne MT, Varnes ME. 1994. Effects of extracellular and intracellular pH on repair of potentially lethal damage, chromosome aberrations and DNA double-strand breaks in irradiated plateau-phase A549 cells. *Radiat. Res.* 139:152–62
28. Massonneau J, Ouellet C, Lucien F, Dubois CM, Tyler J, Boissonneault G. 2018. Suboptimal extracellular pH values alter DNA damage response to induced double-strand breaks. *FEBS Open. Biol.* 8:416–25
29. Pedersen SF, Novak I, Alves F, Schwab A, Pardo LA. 2017. Alternating pH landscapes shape epithelial cancer initiation and progression: focus on pancreatic cancer. *Bioessays* 39:1600253

30. Axelrad JE, Lichtiger S, Yajnik V. 2016. Inflammatory bowel disease and cancer: the role of inflammation, immunosuppression, and cancer treatment. *World J. Gastroenterol.* 22:4794–801
31. Piconese S, Cammarata I, Barnaba V. 2018. Viral hepatitis, inflammation, and cancer: a lesson for autoimmunity. *J. Autoimmun.* 95:58–68
32. Boedtkjer E, Moreira JM, Mele M, Vahl P, Wielenga VT, et al. 2013. Contribution of Na^+,HCO_3^--cotransport to cellular pH control in human breast cancer: a role for the breast cancer susceptibility locus NBCn1 (SLC4A7). *Int. J. Cancer* 132:1288–99
33. Ahmed S, Thomas G, Ghoussaini M, Healey CS, Humphreys MK, et al. 2009. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat. Genet.* 41:585–90
34. Kwon TH, Fulton C, Wang W, Kurtz I, Frokiaer J, et al. 2002. Chronic metabolic acidosis upregulates rat kidney Na^+-HCO_3^- cotransporters NBCn1 and NBC3 but not NBC1. *Am. J. Physiol. Ren. Physiol.* 282:F341–51
35. Kanaan A, Douglas RM, Alper SL, Boron WF, Haddad GG. 2007. Effect of chronic elevated carbon dioxide on the expression of acid-base transporters in the neonatal and adult mouse. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293:R1294–302
36. Rosenberg HKML. 2010. *Regulation of electroneutral Na^+,HCO_3^--cotransporter NBCn1*. PhD Diss., Aarhus Univ.
37. Gorbatenko A, Olesen CW, Loebl N, Sigurdsson HH, Bianchi C, et al. 2016. Oncogenic p95HER2 regulates Na^+–HCO_3^- cotransporter NBCn1 mRNA stability in breast cancer cells via 3′UTR-dependent processes. *Biochem. J.* 473:4027–44
38. McBrian MA, Behbahan IS, Ferrari R, Su T, Huang TW, et al. 2013. Histone acetylation regulates intracellular pH. *Mol. Cell* 49:310–21
39. Corbet C, Draoui N, Polet F, Pinto A, Drozak X, et al. 2014. The SIRT1/HIF2α axis drives reductive glutamine metabolism under chronic acidosis and alters tumor response to therapy. *Cancer Res.* 74:5507–19
40. Zhou ZH, Wang QL, Mao LH, Li XQ, Liu P, et al. 2019. Chromatin accessibility changes are associated with enhanced growth and liver metastasis capacity of acid-adapted colorectal cancer cells. *Cell Cycle* 18:511–22
41. Pavlova NN, Thompson CB. 2016. The emerging hallmarks of cancer metabolism. *Cell Metab.* 23:27–47
42. Corbet C, Pinto A, Martherus R, de Jesus JPS, Polet F, Feron O. 2018. Acidosis drives the reprogramming of fatty acid metabolism in cancer cells through changes in mitochondrial and histone acetylation. *Cell Metab.* 24:311–23
43. Nadtochiy SM, Schafer X, Fu D, Nehrke K, Munger J, Brookes PS. 2016. Acidic pH is a metabolic switch for 2-hydroxyglutarate generation and signaling. *J. Biol. Chem.* 291:20188–97
44. Chen JL, Lucas JE, Schroeder T, Mori S, Wu J, et al. 2008. The genomic analysis of lactic acidosis and acidosis response in human cancers. *PLOS Genet.* 4:e1000293
45. LaMonte G, Tang X, Chen JL, Wu J, Ding CK, et al. 2013. Acidosis induces reprogramming of cellular metabolism to mitigate oxidative stress. *Cancer Metab.* 1:23
46. Kondo A, Yamamoto S, Nakaki R, Shimamura T, Hamakubo T, et al. 2017. Extracellular acidic pH activates the sterol regulatory element-binding protein 2 to promote tumor progression. *Cell Rep.* 18:2228–42
47. Silberman A, Goldman O, Assayag OB, Jacob A, Rabinovich S, et al. 2019. Acid-induced downregulation of ASS1 contributes to the maintenance of intracellular pH in cancer. *Cancer Res.* 79:518–33
48. Intlekofer AM, Wang B, Liu H, Shah H, Carmona-Fontaine C, et al. 2017. L-2-hydroxyglutarate production arises from noncanonical enzyme function at acidic pH. *Nat. Chem. Biol.* 13:494–500
49. Robergs RA, Ghiasvand F, Parker D. 2004. Biochemistry of exercise-induced metabolic acidosis. *Am. J. Physiol. Reg. Int. Physiol.* 287:R502–16
50. Fidelman ML, Seeholzer SH, Walsh KB, Moore RD. 1982. Intracellular pH mediates action of insulin on glycolysis in frog skeletal muscle. *Am. J. Physiol.* 242:C87–93
51. Trivedi B, Danforth WH. 1966. Effect of pH on the kinetics of frog muscle phosphofructokinase. *J. Biol. Chem.* 241:4110–12

52. Le Floch R, Chiche J, Marchiq I, Naiken T, Ilc K, et al. 2011. CD147 subunit of lactate/H^+ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. *PNAS* 108:16663–68
53. Boedtkjer E. 2019. Na^+,HCO_3^- cotransporter NBCn1 accelerates breast carcinogenesis. *Cancer Metastasis Rev*. 38:165–78
54. Gorbatenko A, Olesen CW, Boedtkjer E, Pedersen SF. 2014. Regulation and roles of bicarbonate transporters in cancer. *Front. Physiol.* 5:130
55. Parks SK, Chiche J, Pouyssegur J. 2013. Disrupting proton dynamics and energy metabolism for cancer therapy. *Nat. Rev. Cancer* 13:611–23
56. Pouyssegur J, Franchi A, Pages G. 2001. pH_i, aerobic glycolysis and vascular endothelial growth factor in tumour growth. *Novartis Found. Symp.* 240:186–96
57. Lee S, Axelsen TV, Jessen N, Pedersen SF, Vahl P, Boedtkjer E. 2018. Na^+,HCO_3^--cotransporter NBCn1 (Slc4a7) accelerates ErbB2-induced breast cancer development and tumor growth in mice. *Oncogene* 37:5569–84
58. Franchi A, Silvestre P, Pouysségur J. 1981. A genetic approach to the role of energy metabolism in the growth of tumor cells: tumorigenicity of fibroblast mutants deficient either in glycolysis or in respiration. *Int. J. Cancer* 27:819–27
59. Vander Heiden MG, Cantley LC, Thompson CB. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324:1029–33
60. San-Millan I, Brooks GA. 2017. Reexamining cancer metabolism: lactate production for carcinogenesis could be the purpose and explanation of the Warburg Effect. *Carcinogenesis* 38:119–33
61. Dhup S, Dadhich RK, Porporato PE, Sonveaux P. 2012. Multiple biological activities of lactic acid in cancer: influences on tumor growth, angiogenesis and metastasis. *Curr. Pharm. Des.* 18:1319–30
62. Wilde BR, Ye Z, Lim TY, Ayer DE. 2019. Cellular acidosis triggers human MondoA transcriptional activity by driving mitochondrial ATP production. *eLife* 8:e40199
63. Khacho M, Tarabay M, Patten D, Khacho P, MacLaurin JG, et al. 2014. Acidosis overrides oxygen deprivation to maintain mitochondrial function and cell survival. *Nat. Commun.* 5:3550
64. Wu H, Ying M, Hu X. 2016. Lactic acidosis switches cancer cells from aerobic glycolysis back to dominant oxidative phosphorylation. *Oncotarget* 7:40621–29
65. Hardee ME, Dewhirst MW, Agarwal N, Sorg BS. 2009. Novel imaging provides new insights into mechanisms of oxygen transport in tumors. *Curr. Mol. Med.* 9:435–41
66. Vartanian A, Singh SK, Agnihotri S, Jalali S, Burrell K, et al. 2014. GBM's multifaceted landscape: highlighting regional and microenvironmental heterogeneity. *Neuro-Oncol.* 16:1167–75
67. Hensley CT, Faubert B, Yuan Q, Lev-Cohain N, Jin E, et al. 2016. Metabolic heterogeneity in human lung tumors. *Cell* 164:681–94
68. Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, et al. 2008. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *J. Clin. Investig.* 118:3930–42
69. Fox CJ, Hammerman PS, Thompson CB. 2005. Fuel feeds function: energy metabolism and the T-cell response. *Nat. Rev. Immunol.* 5:844–52
70. Maciver NJ, Jacobs SR, Wieman HL, Wofford JA, Coloff JL, Rathmell JC. 2008. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J. Leukoc. Biol.* 84:949–57
71. Altman BJ, Stine ZE, Dang CV. 2016. From Krebs to clinic: glutamine metabolism to cancer therapy. *Nat. Rev. Cancer* 16:619–34
72. Currie E, Schulze A, Zechner R, Walther TC, Farese RV Jr. 2013. Cellular fatty acid metabolism and cancer. *Cell Metab.* 18:153–61
73. Barba I, Cabañas ME, Arus C. 1999. The relationship between nuclear magnetic resonance-visible lipids, lipid droplets, and cell proliferation in cultured C6 cells. *Cancer Res.* 59:1861–8
74. Menard JA, Christianson HC, Kucharzewska P, Bourseau-Guilmain E, Svensson KJ, et al. 2016. Metastasis stimulation by hypoxia and acidosis-induced extracellular lipid uptake is mediated by proteoglycan-dependent endocytosis. *Cancer Res.* 76:4828–40

75. Mackenzie CG, Mackenzie JB, Beck P. 1961. The effect of pH on growth, protein synthesis, and lipid-rich particles of cultured mammalian cells. *J. Biophys. Biochem. Cytol.* 9:141–56
76. Shyu P Jr., Wong XFA, Crasta K, Thibault G. 2018. Dropping in on lipid droplets: insights into cellular stress and cancer. *Biosci. Rep.* 38:BSR20180764
77. Marino ML, Pellegrini P, Di Lernia G, Djavaheri-Mergny M, Brnjic S, et al. 2012. Autophagy is a protective mechanism for human melanoma cells under acidic stress. *J. Biol. Chem.* 287:30664–76
78. Wojtkowiak JW, Rothberg JM, Kumar V, Schramm KJ, Haller E, et al. 2012. Chronic autophagy is a cellular adaptation to tumor acidic pH microenvironments. *Cancer Res.* 72:3938–47
79. Xie WY, Zhou XD, Li Q, Chen LX, Ran DH. 2015. Acid-induced autophagy protects human lung cancer cells from apoptosis by activating ER stress. *Exp. Cell Res.* 339:270–79
80. Glunde K, Guggino SE, Solaiyappan M, Pathak AP, Ichikawa Y, Bhujwalla ZM. 2003. Extracellular acidification alters lysosomal trafficking in human breast cancer cells. *Neoplasia* 5:533–45
81. Steffan JJ, Snider JL, Skalli O, Welbourne T, Cardelli JA. 2009. Na^+/H^+ exchangers and RhoA regulate acidic extracellular pH-induced lysosome trafficking in prostate cancer cells. *Traffic* 10:737–53
82. Damaghi M, Tafreshi NK, Lloyd MC, Sprung R, Estrella V, et al. 2015. Chronic acidosis in the tumour microenvironment selects for overexpression of LAMP2 in the plasma membrane. *Nat. Commun.* 6:8752
83. Giroux V, Rustgi AK. 2017. Metaplasia: tissue injury adaptation and a precursor to the dysplasia-cancer sequence. *Nat. Rev. Cancer* 17:594–604
84. Flinck M, Kramer SH, Pedersen SF. 2018. Roles of pH in control of cell proliferation. *Acta Physiol.* 223:e13068
85. Putney LK, Barber DL. 2003. Na-H exchange-dependent increase in intracellular pH times G_2/M entry and transition. *J. Biol. Chem.* 278:44645–49
86. Flinck M, Kramer SH, Schnipper J, Andersen AP, Pedersen SF. 2018. The acid-base transport proteins NHE1 and NBCn1 regulate cell cycle progression in human breast cancer cells. *Cell Cycle* 17:1056–67
87. Rotin D, Steele-Norwood D, Grinstein S, Tannock I. 1989. Requirement of the Na^+/H^+ exchanger for tumor growth. *Cancer Res.* 49:205–11
88. Huang WC, Swietach P, Vaughan-Jones RD, Ansorge O, Glitsch MD. 2008. Extracellular acidification elicits spatially and temporally distinct Ca^{2+} signals. *Curr. Biol.* 18:781–85
89. Wiley SZ, Sriram K, Salmerón C, Insel PA. 2019. GPR68: an emerging drug target in cancer. *Int. J. Mol. Sci.* 20:559
90. Zhou ZH, Song JW, Li W, Liu X, Cao L, et al. 2017. The acid-sensing ion channel, ASIC2, promotes invasion and metastasis of colorectal cancer under acidosis by activating the calcineurin/NFAT1 axis. *J. Exp. Clin. Cancer Res.* 36:130
91. Jenkins GJ, D'Souza FR, Suzen SH, Eltahir ZS, James SA, et al. 2007. Deoxycholic acid at neutral and acid pH, is genotoxic to oesophageal cells through the induction of ROS: the potential role of anti-oxidants in Barrett's oesophagus. *Carcinogenesis* 28:136–42
92. Grillo-Hill BK, Choi C, Jimenez-Vidal M, Barber DL. 2015. Increased H^+ efflux is sufficient to induce dysplasia and necessary for viability with oncogene expression. *eLife* 4:e03270
93. Jensen HH, Pedersen GA, Morgen JJ, Parsons M, Pedersen SF, Nejsum LN. 2019. The Na^+/H^+ exchanger NHE1 localizes as clusters to cryptic lamellipodia and accelerates collective epithelial cell migration. *J. Physiol.* 597:849–67
94. Bernards R, Weinberg RA. 2002. Metastasis genes: a progression puzzle. *Nature* 418:823
95. Moellering RE, Black KC, Krishnamurty C, Baggett BK, Stafford P, et al. 2008. Acid treatment of melanoma cells selects for invasive phenotypes. *Clin. Exp. Metastasis* 25:411–25
96. Tomlinson IP, Novelli MR, Bodmer WF. 1996. The mutation rate and cancer. *PNAS* 93:14800–3
97. Andersen AP, Samsøe-Petersen J, Oernbo EK, Boedtkjer E, Moreira JMA, et al. 2018. The net acid extruders NHE1, NBCn1 and MCT4 promote mammary tumor growth through distinct but overlapping mechanisms. *Int. J. Cancer* 142:2529–42
98. Plaks V, Kong N, Werb Z. 2015. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? *Cell Stem Cell* 16:225–38
99. Hjelmeland AB, Wu Q, Heddleston JM, Choudhary GS, MacSwords J, et al. 2011. Acidic stress promotes a glioma stem cell phenotype. *Cell Death Differ.* 18:829–40

100. Filatova A, Seidel S, Böğürcü N, Gräf S, Garvalov BK, Acker T. 2016. Acidosis acts through HSP90 in a PHD/VHL-independent manner to promote HIF function and stem cell maintenance in glioma. *Cancer Res.* 76:5845–56
101. Hu P, Li S, Tian N, Wu F, Hu Y, et al. 2019. Acidosis enhances the self-renewal and mitochondrial respiration of stem cell-like glioma cells through CYP24A1-mediated reduction of vitamin D. *Cell Death Dis.* 10:25
102. De Los Angeles A, Ferrari F, Fujiwara Y, Mathieu R, Lee S, et al. 2015. Failure to replicate the STAP cell phenomenon. *Nature* 525:E6–9
103. Tian Y, Bresenitz P, Reska A, El Moussaoui L, Beier CP, Grunder S. 2017. Glioblastoma cancer stem cell lines express functional acid sensing ion channels ASIC1a and ASIC3. *Sci. Rep.* 7:13674
104. Ulmschneider B, Grillo-Hill BK, Benitez M, Azimova DR, Barber DL, Nystul TG. 2016. Increased intracellular pH is necessary for adult epithelial and embryonic stem cell differentiation. *J. Cell Biol.* 215:345–55
105. Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H. 2015. Cancer-associated fibroblasts: their characteristics and their roles in tumor growth. *Cancers* 7:2443–58
106. Angelucci C, Maulucci G, Lama G, Proietti G, Colabianchi A, et al. 2012. Epithelial-stromal interactions in human breast cancer: effects on adhesion, plasma membrane fluidity and migration speed and directness. *PLOS ONE* 7:e50804
107. Fiaschi T, Giannoni E, Taddei ML, Cirri P, Marini A, et al. 2013. Carbonic anhydrase IX from cancer-associated fibroblasts drives epithelial-mesenchymal transition in prostate carcinoma cells. *Cell Cycle* 12:1791–801
108. Hulikova A, Black N, Hsia L-T, Wilding J, Bodmer WF, Swietach P. 2016. Stromal uptake and transmission of acid is a pathway for venting cancer cell-generated acid. *PNAS* 113:E5344–53
109. Swietach P, Patiar S, Supuran CT, Harris AL, Vaughan-Jones RD. 2009. The role of carbonic anhydrase 9 in regulating extracellular and intracellular pH in three-dimensional tumor cell growths. *J. Biol. Chem.* 284:20299–310
110. Dovmark TH, Hulikova A, Niederer SA, Vaughan-Jones RD, Swietach P. 2018. Normoxic cells remotely regulate the acid-base balance of cells at the hypoxic core of connexin-coupled tumor growths. *FASEB J.* 32:83–96
111. Dovmark TH, Saccomano M, Hulikova A, Alves F, Swietach P. 2017. Connexin-43 channels are a pathway for discharging lactate from glycolytic pancreatic ductal adenocarcinoma cells. *Oncogene* 36:4538–50
112. Pérez-Escuredo J, Van Hée VF, Sboarina M, Falces J, Payen VL, et al. 2016. Monocarboxylate transporters in the brain and in cancer. *Biochim. Biophys. Acta* 1863:2481–97
113. Guo S, Deng CX. 2018. Effect of stromal cells in tumor microenvironment on metastasis initiation. *Int. J. Biol. Sci.* 14:2083–93
114. Guido C, Whitaker-Menezes D, Capparelli C, Balliet R, Lin Z, et al. 2012. Metabolic reprogramming of cancer-associated fibroblasts by TGF-β drives tumor growth: connecting TGF-β signaling with "Warburg-like" cancer metabolism and L-lactate production. *Cell Cycle* 11:3019–35
115. Vaughan RA, Garcia-Smith R, Dorsey J, Griffith JK, Bisoffi M, Trujillo KA. 2013. Tumor necrosis factor alpha induces Warburg-like metabolism and is reversed by anti-inflammatory curcumin in breast epithelial cells. *Int. J. Cancer* 133:2504–10
116. Vaughan RA, Garcia-Smith R, Trujillo KA, Bisoffi M. 2013. Tumor necrosis factor alpha increases aerobic glycolysis and reduces oxidative metabolism in prostate epithelial cells. *Prostate* 73:1538–46
117. Apostolopoulou M, Ligon L. 2012. Cadherin-23 mediates heterotypic cell-cell adhesion between breast cancer epithelial cells and fibroblasts. *PLOS ONE* 7:e33289
118. Roh-Johnson M, Bravo-Cordero JJ, Patsialou A, Sharma VP, Guo P, et al. 2014. Macrophage contact induces RhoA GTPase signaling to trigger tumor cell intravasation. *Oncogene* 33:4203–12
119. Groebe K, Vaupel P. 1988. Evaluation of oxygen diffusion distances in human breast cancer xenografts using tumor-specific in vivo data: role of various mechanisms in the development of tumor hypoxia. *Int. J. Radiat Oncol. Biol. Phys.* 15:691–97
120. Döme B, Hendrix MJC, Paku S, Tóvári J, Tímár J. 2007. Alternative vascularization mechanisms in cancer: pathology and therapeutic implications. *Am. J. Pathol.* 170:1–15

121. Froelunde AS, Ohlenbusch M, Hansen KB, Jessen N, Kim S, Boedtkjer E. 2018. Murine breast cancer feed arteries are thin-walled with reduced α_{1A}-adrenoceptor expression and attenuated sympathetic vasocontraction. *Breast Cancer Res.* 20:20
122. Voss NCS, Kold-Petersen H, Boedtkjer E. 2019. Enhanced nitric oxide signaling amplifies vasorelaxation of human colon cancer feed arteries. *Am. J. Physiol. Heart Circ. Physiol.* 316:H245–54
123. Jain RK. 2001. Delivery of molecular and cellular medicine to solid tumors. *Adv. Drug Deliv. Rev.* 46:149–68
124. Trédan O, Galmarini CM, Patel K, Tannock IF. 2007. Drug resistance and the solid tumor microenvironment. *J. Natl. Cancer Inst.* 99:1441–54
125. Donnem T, Hu J, Ferguson M, Adighibe O, Snell C, et al. 2013. Vessel co-option in primary human tumors and metastases: an obstacle to effective anti-angiogenic treatment? *Cancer Med.* 2:427–36
126. Katayama Y, Uchino J, Chihara Y, Tamiya N, Kaneko Y, et al. 2019. Tumor neovascularization and developments in therapeutics. *Cancers* 11:316
127. Huang S, He P, Xu D, Li J, Peng X, Tang Y. 2017. Acidic stress induces apoptosis and inhibits angiogenesis in human bone marrow-derived endothelial progenitor cells. *Oncol. Lett.* 14:5695–702
128. Faes S, Uldry E, Planche A, Santoro T, Pythoud C, et al. 2016. Acidic pH reduces VEGF-mediated endothelial cell responses by downregulation of VEGFR-2; relevance for anti-angiogenic therapies. *Oncotarget* 7:86026–38
129. Pedersen AK, de Melo JML, Morup N, Tritsaris K, Pedersen SF. 2017. Tumor microenvironment conditions alter Akt and Na^+/H^+ exchanger NHE1 expression in endothelial cells more than hypoxia alone: implications for endothelial cell function in cancer. *BMC Cancer* 17:542
130. Burbridge MF, West DC, Atassi G, Tucker GC. 1999. The effect of extracellular pH on angiogenesis in vitro. *Angiogenesis* 3:281–88
131. Castañeda-Gill JM, Vishwanatha JK. 2016. Antiangiogenic mechanisms and factors in breast cancer treatment. *J. Carcinog.* 15:1
132. Dass CR, Choong PF. 2008. Cancer angiogenesis: targeting the heel of Achilles. *J. Drug Target.* 16:449–54
133. Stachura J, Wachowska M, Kilarski WW, Güç E, Golab J, Muchowicz A. 2016. The dual role of tumor lymphatic vessels in dissemination of metastases and immune response development. *Oncoimmunology* 5:e1182278
134. Moeller AL, Hjortdal V, Boedtkjer DMB, Boedtkjer E. 2019. Acidosis inhibits rhythmic contractions of human thoracic ducts. *Physiol. Rep.* 7:e14074
135. Lobov GI, Kubyshkina NA. 2001. Effect of acidosis on contractile function of mesenterial lymphatic vessels in bulls. *Bull. Exp. Biol. Med.* 132:622–25
136. Gatenby RA, Gawlinski ET, Gmitro AF, Kaylor B, Gillies RJ. 2006. Acid-mediated tumor invasion: a multidisciplinary study. *Cancer Res.* 66:5216–23
137. Robey IF, Baggett BK, Kirkpatrick ND, Roe DJ, Dosescu J, et al. 2009. Bicarbonate increases tumor pH and inhibits spontaneous metastases. *Cancer Res.* 69:2260–68
138. van den Akker J, Schoorl MJC, Bakker ENTP, vanBavel E. 2010. Small artery remodeling: current concepts and questions. *J. Vasc. Res.* 47:183–202
139. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, et al. 2014. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. *J. Pathol.* 232:199–209
140. Ibrahim-Hashim A, Abrahams D, Enriquez-Navas PM, Luddy K, Gatenby RA, Gillies RJ. 2017. Tris-base buffer: a promising new inhibitor for cancer progression and metastasis. *Cancer Med.* 6:1720–29
141. Pilon-Thomas S, Kodumudi KN, El-Kenawi AE, Russell S, Weber AM, et al. 2016. Neutralization of tumor acidity improves antitumor responses to immunotherapy. *Cancer Res.* 76:1381–90
142. Calcinotto A, Filipazzi P, Grioni M, Iero M, De Milito A, et al. 2012. Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. *Cancer Res.* 72:2746–56
143. Bosticardo M, Ariotti S, Losana G, Bernabei P, Forni G, Novelli F. 2001. Biased activation of human T lymphocytes due to low extracellular pH is antagonized by B7/CD28 costimulation. *Eur. J. Immunol.* 31:2829–38

144. Vermeulen M, Giordano M, Trevani AS, Sedlik C, Gamberale R, et al. 2004. Acidosis improves uptake of antigens and MHC class I-restricted presentation by dendritic cells. *J. Immunol.* 172:3196–204
145. Gottfried E, Kunz-Schughart LA, Ebner S, Mueller-Klieser W, Hoves S, et al. 2006. Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. *Blood* 107:2013–21
146. Nasi A, Fekete T, Krishnamurthy A, Snowden S, Rajnavölgyi E, et al. 2013. Dendritic cell reprogramming by endogenously produced lactic acid. *J. Immunol.* 191:3090–99
147. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, et al. 2016. LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab.* 24:657–71
148. Loeffler DA, Heppner GH, Juneau PL. 1991. Natural killer-cell activity under conditions reflective of tumor micro-environment. *Int. J. Cancer* 48:895–99
149. Jayaprakash P, Ai M, Liu A, Budhani P, Bartkowiak T, et al. 2018. Targeted hypoxia reduction restores T cell infiltration and sensitizes prostate cancer to immunotherapy. *J. Clin. Investig.* 128:5137–49
150. Martinez-Zaguilan R, Seftor EA, Seftor RE, Chu YW, Gillies RJ, Hendrix MJ. 1996. Acidic pH enhances the invasive behavior of human melanoma cells. *Clin. Exp. Metastasis* 14:176–86
151. Estrella V, Chen T, Lloyd M, Wojtkowiak J, Cornnell HH, et al. 2013. Acidity generated by the tumor microenvironment drives local invasion. *Cancer Res.* 73:1524–35
152. Zhu S, Zhou H-Y, Deng S-C, Deng S-J, He C, et al. 2017. ASIC1 and ASIC3 contribute to acidity-induced EMT of pancreatic cancer through activating Ca^{2+}/RhoA pathway. *Cell Death Dis.* 8:e2806
153. Peppicelli S, Bianchini F, Torre E, Calorini L. 2014. Contribution of acidic melanoma cells undergoing epithelial-to-mesenchymal transition to aggressiveness of non-acidic melanoma cells. *Clin. Exp. Metastasis* 31:423–33
154. Boussadia Z, Lamberti J, Mattei F, Pizzi E, Puglisi R, et al. 2018. Acidic microenvironment plays a key role in human melanoma progression through a sustained exosome mediated transfer of clinically relevant metastatic molecules. *J. Exp. Clin. Cancer Res.* 37:245
155. Hofschröer V, Koch KA, Ludwig FT, Friedl P, Oberleithner H, et al. 2017. Extracellular protonation modulates cell-cell interaction mechanics and tissue invasion in human melanoma cells. *Sci. Rep.* 7:42369
156. Defamie N, Chepied A, Mesnil M. 2014. Connexins, gap junctions and tissue invasion. *FEBS Lett.* 588:1331–38
157. Boedtkjer E, Kim S, Aalkjaer C. 2013. Endothelial alkalinisation inhibits gap junction communication and endothelium-derived hyperpolarisations in mouse mesenteric arteries. *J. Physiol.* 591:1447–61
158. Swietach P, Rossini A, Spitzer KW, Vaughan-Jones RD. 2007. H^+ ion activation and inactivation of the ventricular gap junction: a basis for spatial regulation of intracellular pH. *Circ. Res.* 100:1045–54
159. Spray DC, Harris AL, Bennett MV. 1981. Gap junctional conductance is a simple and sensitive function of intracellular pH. *Science* 211:712–15
160. Busco G, Cardone RA, Greco MR, Bellizzi A, Colella M, et al. 2010. NHE1 promotes invadopodial ECM proteolysis through acidification of the peri-invadopodial space. *FASEB J.* 24:3903–15
161. Kong SC, Nohr-Nielsen A, Zeeberg K, Reshkin SJ, Hoffmann EK, et al. 2016. Monocarboxylate transporters MCT1 and MCT4 regulate migration and invasion of pancreatic ductal adenocarcinoma cells. *Pancreas* 45:1036–47
162. Bourguignon LY, Singleton PA, Diedrich F, Stern R, Gilad E. 2004. CD44 interaction with Na^+-H^+ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B activation and breast tumor cell invasion. *J. Biol. Chem.* 279:26991–7007
163. Cotter K, Capecci J, Sennoune S, Huss M, Maier M, et al. 2015. Activity of plasma membrane V-ATPases is critical for the invasion of MDA-MB231 breast cancer cells. *J. Biol. Chem.* 290:3680–92
164. Brisson L, Reshkin SJ, Gore J, Roger S. 2012. pH regulators in invadosomal functioning: proton delivery for matrix tasting. *Eur. J. Cell Biol.* 91:847–60
165. Bailey KM, Wojtkowiak JW, Cornnell HH, Ribeiro MC, Balagurunathan Y, et al. 2014. Mechanisms of buffer therapy resistance. *Neoplasia* 16:354–64
166. Boedtkjer E, Bentzon JF, Dam VS, Aalkjaer C. 2016. Na^+,HCO_3^--cotransporter NBCn1 increases pH_i gradients, filopodia and migration of smooth muscle cells and promotes arterial remodeling. *Cardiovasc. Res.* 111:227–39

167. Schwab A, Rossmann H, Klein M, Dieterich P, Gassner B, et al. 2005. Functional role of Na^+-HCO_3^- cotransport in migration of transformed renal epithelial cells. *J. Physiol.* 568:445–58
168. Stock C, Mueller M, Kraehling H, Mally S, Noel J, et al. 2007. pH nanoenvironment at the surface of single melanoma cells. *Cell Physiol. Biochem.* 20:679–86
169. Harrison RK, Chang B, Niedzwiecki L, Stein RL. 1992. Mechanistic studies on the human matrix metalloproteinase stromelysin. *Biochemistry* 31:10757–62
170. Srivastava J, Barreiro G, Groscurth S, Gingras AR, Goult BT, et al. 2008. Structural model and functional significance of pH-dependent talin-actin binding for focal adhesion remodeling. *PNAS* 105:14436–41