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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**).
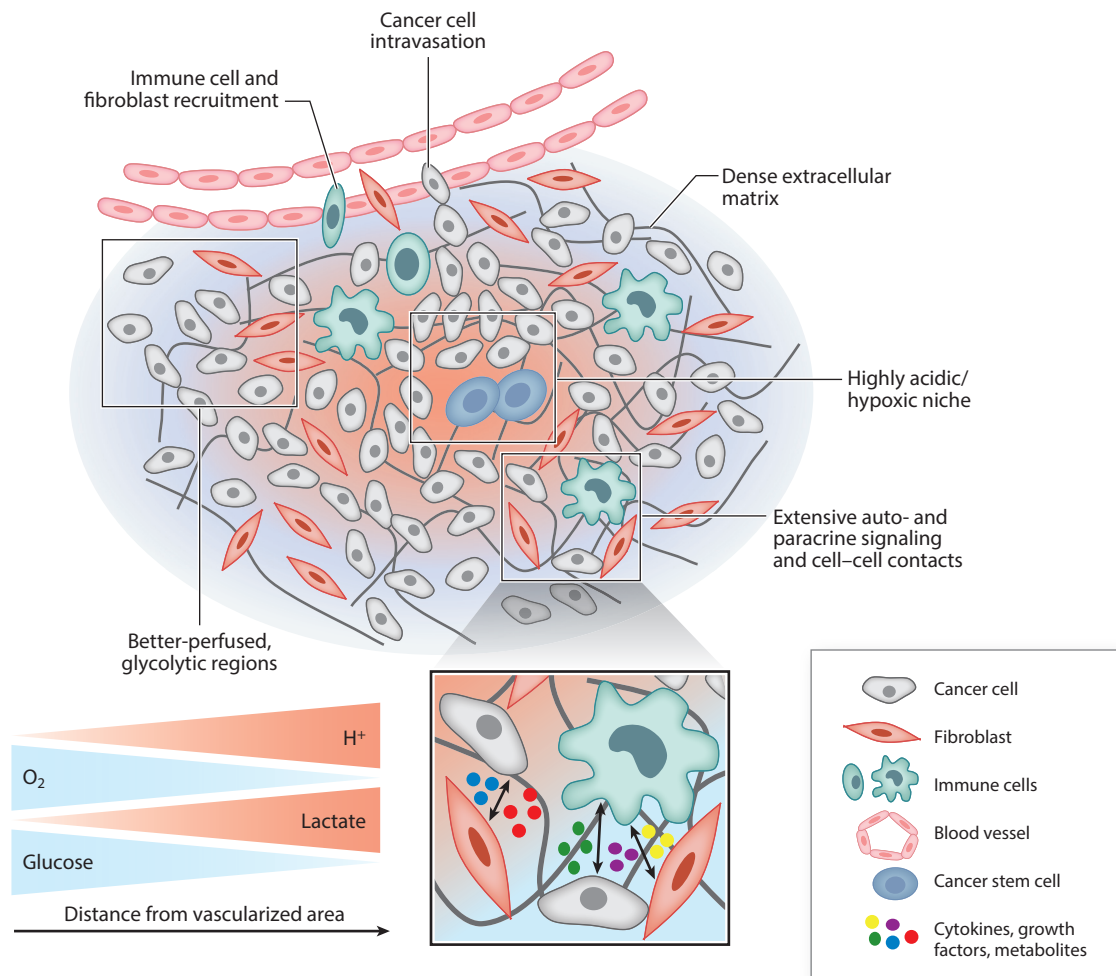
Figure 1

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
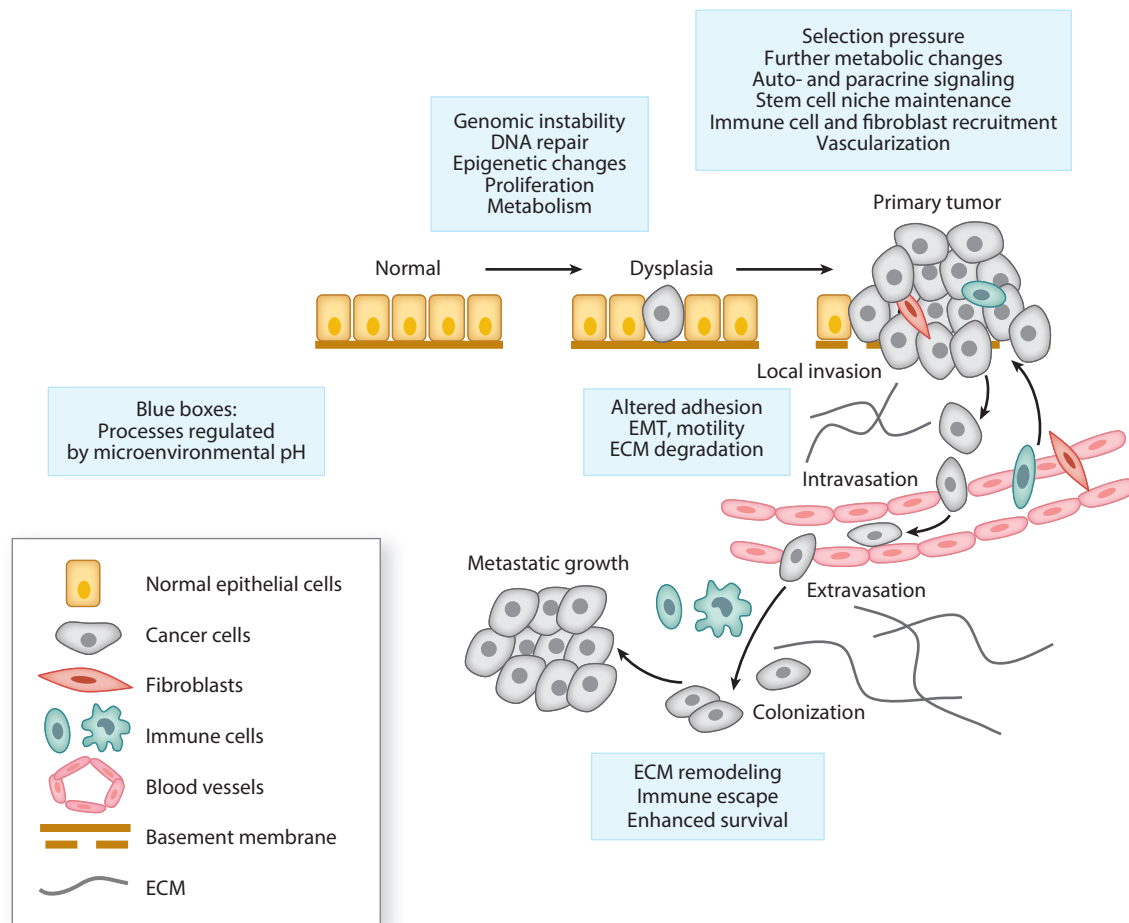
Figure 2

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.
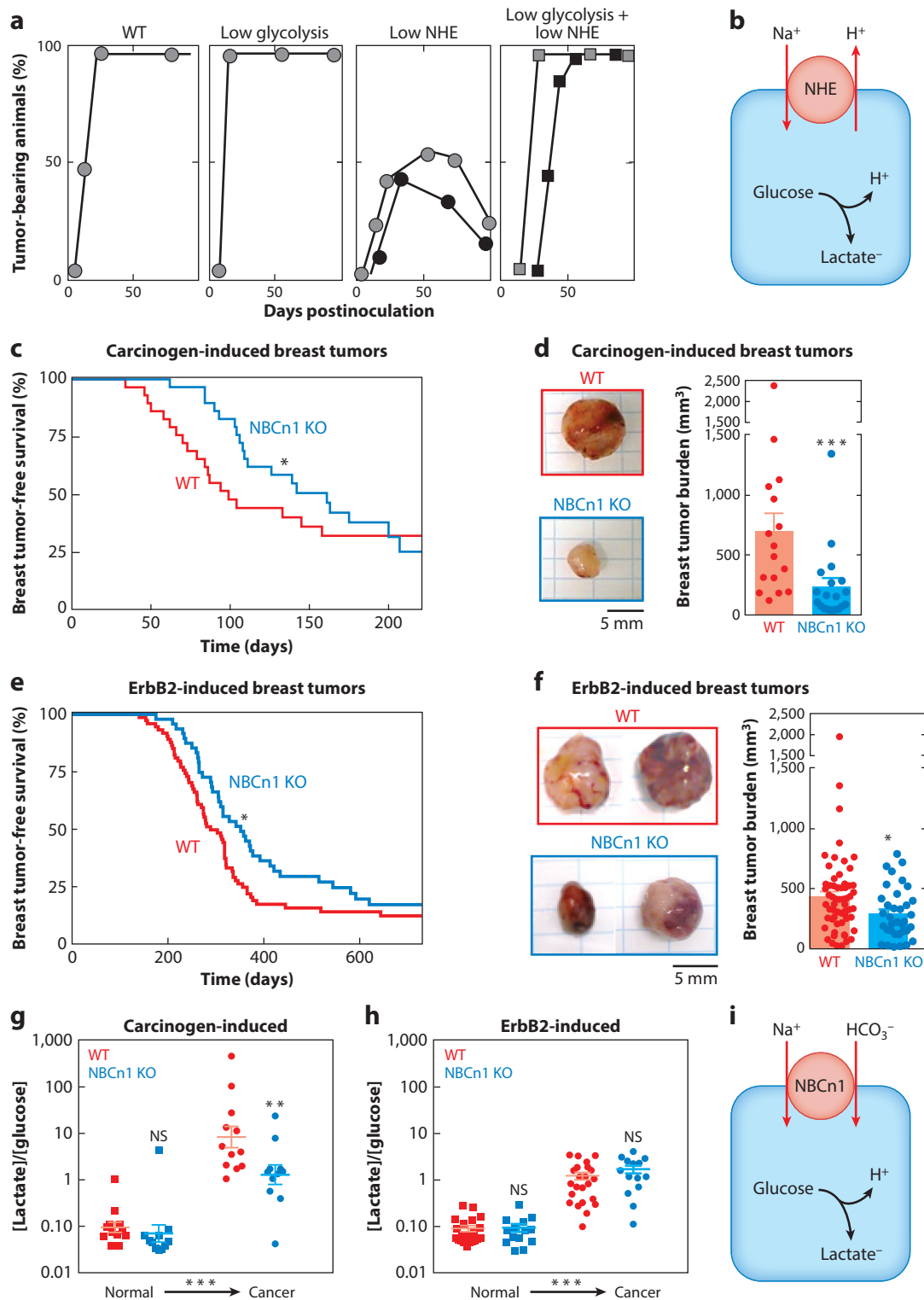
Oxidative Phosphorylation and Glycolysis

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 3a,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 3c,d**), and glycolysis (**Figure 3g**), as evaluated on the basis of the interstitial [lactate] to [glucose] ratio (8). The lower glycolytic activity (**Figure 3g**) may explain, but could also be a consequence of, the smaller tumor size (**Figure 3d**) in the NBCn1 knockout mice (8). In tumors developing due to breast epithelial overexpression of ErbB2 (57), NBCn1 knockout still delays tumor development (**Figure 3e**) and decelerates tumor growth (**Figure 3f**) although the effect is of smaller magnitude and is not associated with changes in glycolysis (**Figure 3h**). In mice with intact NBCn1 expression [compare wild type (WT) in **Figure 3g** to WT in **Figure 3h**], 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 3c–i**) is consistent with previous xenograft studies (**Figure 3a,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

a

WT | Low glycolysis | Low NHE | Low glycolysis + low NHE

Tumor-bearing animals (%)

Days postinoculation

b

Na$^+$ H$^+$

NHE

Glucose → H$^+$

Lactate$^-$

c

Carcinogen-induced breast tumors

Breast tumor-free survival (%)

NBCn1 KO

WT

*

Time (days)

d

Carcinogen-induced breast tumors

WT

NBCn1 KO

5 mm

Breast tumor burden (mm^3)

***

WT   NBCn1 KO

e

ErbB2-induced breast tumors

Breast tumor-free survival (%)

NBCn1 KO

WT

*

Time (days)

f

ErbB2-induced breast tumors

WT

NBCn1 KO

5 mm

Breast tumor burden (mm^3)

*

WT   NBCn1 KO

g

Carcinogen-induced

WT
NBCn1 KO

[Lactate]/[glucose]

NS

**

Normal   *** → Cancer

h

ErbB2-induced

WT
NBCn1 KO

[Lactate]/[glucose]

NS

NS

Normal   *** → Cancer

i

Na$^+$ HCO$_3^-$

NBCn1

Glucose → H$^+$

Lactate$^-$

(*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

ACKNOWLEDGMENTS

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