pH in Cancer: Emerging Concepts

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Target audience: – Clinical and Pre-clinical cancer researchers
Objectives: –
1. Understand the causes of tumor acidity
2. Be able to discuss some consequences of acidity that are relevant to carcinogenesis and therapy.
3. Discuss some of the approaches to measure Tumor pH with MRS, MRI, optical or PET methods.

Causes and Consequence of tumor acidity

Two common characteristics of malignant solid tumors is a high rate of glucose metabolism, usually measured with FDG PET, and heterogeneous perfusion, usually measured with Contrast Enhanced MRI. The combination of these two characteristics leads to microenvironments that are hypoxic and acidic. It can also be shown that these characteristics are present early in carcinogenesis, and they are significant factors in guiding the somatic evolution of cancers. Conversion of a normal epithelium to a metastatic carcinoma occurs through a series of steps along an evolutionary trajectory and hence represents the successful responses to sequential environmental selections. Commonly observed traits, or "hallmarks" (1), of cancer represent successful adaptation strategies to commonly experienced environmental selection pressures, including hypoxia and acidosis, which are not only highly selective, they can be shown to be genotoxic (2, 3). In addition to promoting evolutionary divergence in cancers, acid pH can also inhibit the activity of numerous chemotherapeutic agents(4).

Imaging tumor pH

Interest in measuring tumor pH has a long history, and has been reviewed in (5, 6). Early measurements used radioactive weak acid tracers, and pH-sensitive microelectrodes. In the 1980's and 1990's, ^{31}P MRS showed that the intracellular pHi of tumors was neutral-to-alkaline and that the extracellular pHe was acidic (7, 8). ^{31}P MRS has limited spatial resolution and higher resolution can be obtained with ^{1}H and ^{19}F labeled agents which have been used to measure localized tumor pH using spectroscopic imaging (MRSI) with spatial resolution approaching 1x1x1 mm (9). These images show significant pH heterogeneity within tumors. Higher resolution pH measurements can be obtained with pH-dependent T1 relaxometry (10, 11). An issue with these measurements is the need for simultaneous pixel-by-pixel correction for concentration. An alternative method uses the rate of acid-catalyzed chemical exchange of hydrogens with bulk water to measure pH. This approach, chemical exchange saturation transfer (CEST), can be made more sensitive using pH-sensitive paramagnetic lanthanide chelates (ParaCEST) or through a large number of exchanging groups (DiaCEST) so that measurements can be made with clinically achievable doses. A related magnetization transfer approach interrogates the pH-dependent exchange of hydrogen with endogenous amides of peptide backbones. This technique, called Amide Proton Transfer, APT, has been successfully used in humans (12).

Emerging Concept – Habitat Imaging

Although there are a broad portfolio of approaches with which to measure pHe in animal tumors, there are no applications at present that allow routine measurement or estimation of pHe within human patients. One approach to this goal is so-called "habitat imaging", wherein functional and molecular images that contain orthogonal information are analyzed to yield specific regions that contain unique combinations of features (27). We have proposed that "hypoxic habitats" should result from deficits in perfusion measured with contrast-enhanced MRI, and should have high cell density and be adjacent to necrotic volumes, as measured by diffusion MRI. Similarly, for acidic pHe voxels, there should be a relative deficit of contrast, combined with a high uptake of 18-FDGby co-registered PET.

Emerging Concept – Metabolic compartmentation

In the past, the existence of an acidic extracellular pH was believed to be simply a mismatch between metabolism and perfusion. An emerging concept in cancer biology is that tumor metabolism is compartmentalized so that cells in

different regions ("habitats") co-evolve different metabolic strategies to maximize their survival. One example of such an adaptation is the observation that cells within the same tumor can be "metabolically symbiotic", in that some fermentative and oxidative cells produce and consume lactate, respectively (13). While originally believed that oxidative cells were primarily stromal in origin (14), it is now known that the system can be more complex, wherein different cancer or stromal cells can adopt either phenotype, depending on their habitat and evolutionary trajectory (15-17). The monocarboxylate transporters, MCT1 and MCT4, are lactate-proton symporters and biomarkers for these metabolic phenotypes, as they import and export lactate, respectively (18). Such metabolic compartmentation is intimately involved in tumor pHe regulation through complex mechanisms.

Emerging Concept – Pseudohypoxia and the Warburg Effect
The Warburg Effect is also known as aerobic glycolysis", and described the constitutive fermentation of glucose even under normoxic conditions. Pet studies have clearly shown a relationship between excessive glucose consumption and poor prognosis(19). Over the last decades, there have been multiple attempts to explain and understand the prevalence of the WE in cancers, reviewed in (20). Regardless of mechanism, it is clear that the WE represents an adaptation called "pseudohypoxia", wherein cells continue to express hypoxia-related proteins (HRPs) even in the presence of normoxia. Further its prevalence in cancer explicitly indicates that the consequences of elevated fermentation provide a survival advantage to cancer cells as the evolve(21). These consequences can be provision of carbons for anabolism, acidifying the extracellular pH, or providing an energy supply under anaerobic conditions. An emerging concept has proposed that aerobic glycolysis is fundamentally needed to provide a rapid temporal response of ATP production in the face of unexpected and rapid demands. This concept is unique in that it considers aerobic glycolysis to be a phyeioligical response to energy demand at the membrane.

Emerging Concept – pHe sensing
Although the extracellular pH of tumors is acidic, many of the cellular responses to this are intracellular. This is a conundrum because the intracellular pH is highly regulated by a portfolio of redundant pH regulatory systems, such as Carbonic Anhydrases; V-ATPase; Na^+/HCO_3^- co-transporters; the Na^+-driven Cl^-/HCO_3^- exchanger (SLC4A8); the monocarboxylate transporters; Na^+/H^+ exchanger 1, NHE1 (SLC9A1); and the anion exchangers AE1-2, (SLC4A1-3). Over the last decade, however, a number of pH-sensing membrane transporters have been identified with external facing histidines whose protonation status affects their behavior. These include two broad categories: G-protein coupled receptors, GPCRs, and acid-stimulated ion channels, ASICs.   The acid-sensitive GPCrs include GPR4, OGR1 (GPR68), TDAG8 (GPR65), and G2A (GPR132), which have also been identified as receptors for lysolipids; sphingosylphosphorylcholine (SPC), lysophosphatidylcholine (LPC) and psychosine (galactosylsphingosine). The ASICs include 7 proteins from 4 genes. It has been shown that ASIC1 and ASIC2 (also known as the capsaicin receptor) are important in cancer-related pain processing

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