Clinical Cancer Research

Imaging Tumor Acidity: pH-Low Insertion Peptide Probe for Optoacoustic Tomography

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Optoacoustic tomography has been used for the detection of pancreatic ductal adenocarcinoma targeted by pH-low insertion peptide (pHLIP) conjugated to near-infrared fluorescent dye. It was proved that tumor targeting is pH dependent. The approach could have major implication for detection and monitoring of pancreatic and other cancers. *Clin Cancer Res; 21(20); 4502–4. ©2015 AACR.*

See related article by Kimbrough et al., p. 4576

In this issue of *Clinical Cancer Research*, Kimbrough and colleagues (1) report that pH-sensitive pHLIP variant 7 (V7) conjugated to near-infrared fluorescent dye, Alexa750, targets pancreatic ductal adenocarcinoma (PDAC) in human S2VP10 and S2013 pancreatic cancer xenograft mouse models with minimal off-target accumulation. At the same time, pH-insensitive K7 pHLIP, where a single protonatable Glu residue was replaced by a positively charged Lys residue, served as a control and showed about 100 times less tumor accumulation. Immediately prior to injection of the constructs, as well as 4 and 24 hours after injection, mice were imaged by using multispectral optoacoustic tomography (MSOT). Optoacoustic imaging is an emerging new technology with the potential to increase sensitivity and improve spatial resolution. It represents a hybrid technique that incorporates advantageous properties of both light and sound, because resolution of the optical contrast obeys the rules of ultrasonic diffraction, rendering photon scattering irrelevant to image resolution (2, 3). Thus, high resolution at depth of organ or tissue could be achieved, in contrast to optical imaging, which is critical for the imaging of tumors in humans.

To improve cancer detection, it is very important to introduce new imaging modalities, which should be accompanied with the development of novel tumor-specific molecular contrast probes. There are a number of ways of tumor targeting; one of the most popular is based on targeting of overexpressed protein biomarkers. However, the genetic instability and consequent heterogeneity of cancer cells limit approaches for treatment of tumors by targeting specific biomarkers, because clonal selection leads cell populations to overcome the therapy by growth of subpopulations that do not express sensitizing levels of the biomarker in question. Cytotoxic therapies, while treating the majority of cancer cells, may spare multidrug-resistant clones, leading to tumor relapse and treatment failure (4). Moreover, this transient depopulation of sensitive tumor cells by chemotherapeutic agents may provide a growth advantage to the surviving cells, leading to outgrowth of resistant clones (5). It is therefore important to develop alternative approaches, which are based on targeting of the tumor microenvironment that is less likely to be subject to resistant selection. One such property is the acidity that is associated with tumor growth and development, which could serve as a universal marker for targeting. Adaptations to the highly acidic microenvironment are critical steps in the transition from an avascular preinvasive tumor to a malignant invasive carcinoma. Although the acidity also varies within and among tumors, the general variation favors therapy, because more aggressive and more metastatic tumors tend to be more acidic (6).

The promise of exploiting tumor acidosis as a cancer biomarker has not been fully realized in clinical practice, even though the acidity has been a known property since the work of Otto Warburg nearly a century ago. The problem has been to find a practical way to target acidity, because the bulk extracellular pH in diseased tissue is just 0.5–0.8 pH units lower than the extracellular pH in healthy tissue (7). From a biologic standpoint, the change is significant and alters the functions and survival of cells. At the same time, from a chemical standpoint, the change is small; therefore, very precise tuning of chemical properties would be needed in a targeting agent. However, it is important to outline that the pH is lowest at the surfaces of cells compared with the bulk extracellular pH and increases with distance from cellular membrane, becoming normal in the vicinity of blood vessels. Therefore, the average pH in tissue is less informative than the pH at cellular surfaces, which needs to be the main target for the development of pH-sensitive agents.

One of the unique approaches introduced for the targeting of tumor acidity is based on the biophysical principles of pH-dependent interaction of a polypeptide with lipid bilayer of a membrane. pH-low insertion peptides are capable of undergoing a pH-induced membrane-associated folding (8), which results in targeting of cancer cells in acidic tumors with minimal accumulation in healthy tissue with normal extracellular pH (Fig. 1). pHLIP peptides possess dual delivery capabilities, making use of the energy of folding to translocate polar cargo molecules across the phospholipid bilayer of a membrane and/or tether molecules to the cell surface. Also, the process of peptide folding within a membrane ensures a high cooperativity of the transition, which cannot be achieved by simple diffusion. Because pHLIPs are in

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doi: 10.1158/1078-0432.CCR-15-1502

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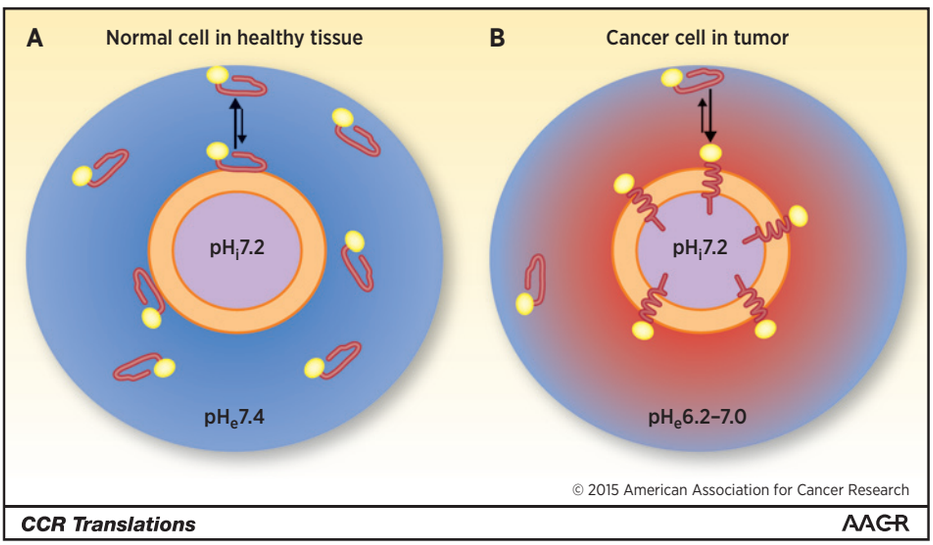

Figure 1.
Schematic presentation of pHLIP peptide (red) conjugated with fluorescent dye (yellow) interaction with plasma membrane of a normal cell in healthy tissue (A) and a cancer cell in tumor (B). The extracellular pH in healthy tissue is around pH7.4. On the other hand, pH gradient exists near the surface of cancer cells: The pH near the plasma membrane is lowest (around pH6.0–6.2), increasing with distance from the membrane. pHLIP peptides are weakly bound to the surface of the cell membrane in healthy tissue. However, at low extracellular pH in tumors, pHLIPs insert into the plasma membrane, tethering imaging probe to cancer cells. In tumors, the equilibrium is shifted toward membrane-inserted form of the peptide, leading to accumulation of the peptide and an imaging agent within a tumor. At the same time, in healthy tissue, the equilibrium is shifted toward membrane non-bound form of the peptide, which results in washing of the peptide from healthy tissue.

equilibrium between membrane bound and non-bound configurations at normal pH, they are capable of sensing pH at the cell surface. As soon as pH drops (even on a half of pH unit), the Asp and Glu residues are protonated, and affinity of peptides to membrane is enhanced dramatically, which triggers folding in the membrane and release of energy. Depending on the pHLIP sequence, protonatable residues could be differently located on the membrane surface, which directly affects the rate of the protonation events at various pHs, and thus pK of peptides insertion into the membrane. The family of pHLIP peptides with pK of insertion varying from 4.5 to 6.5 was introduced (9), and it was confirmed that tumor targeting is indeed pH dependent (10, 11). pHLIPs variant 7 (V7) and variant 3 (V3) were selected as lead candidates for pH-specific delivery of imaging and therapeutic agents to tumors of different origins. It was shown that the fluorescent pHLIPs can localize and specifically detect PDAC in human xenografts as well as PDAC and PanIN lesions in genetically engineered mouse models (12).

Kimbrough and colleagues also used pHLIP variant 7 for the targeting of PDAC and demonstrated that precise mapping of tumors could be achieved by optoacoustic imaging. PDAC remains highly lethal because of its advanced stage at presentation. The lack of specific symptoms (due to the physical position of the organ), and the lack of sensitive and specific biomarkers, make obtaining a diagnosis difficult at an early stage. For these reasons, there is an urgent need for tools to aid in the early and specific detection of PDAC prior to the development of micrometastatic disease. The molecular imaging with targeted probes could potentially improve the early diagnosis, staging, and monitoring of PDAC. Endoscopic, laparoscopic, or handheld applications of MSOT in combination with pH-sensitive pHLIP probes, could aid in the detection and staging of pancreatic tumors, help determine resectability, assist in the identification of viable tumor during surgical intervention, as well as help monitor responses to treatment.

Translation of pHLIP technology and MSOT imaging to clinics may lead to improvements in imaging, treatment, and monitoring outcomes of therapy for tumors of various origins, especially highly aggressive and metastatic ones.

Disclosure of Potential Conflicts of Interest

Y.K. Reshetnyak is an uncompensated President of and has ownership interest in pHLIP, Inc. No other potential conflicts of interest were disclosed.

Grant Support

Y.K. Reshetnyak is supported by the NCI of the NIH under award number CA174413 and the National Institute of General Medical Sciences of the NIH under award number GM073857.

Received July 9, 2015; accepted July 13, 2015; published OnlineFirst July 29, 2015.

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