Physics Faculty Publications

Physics

7-13-2015

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

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Abstract

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 a pH-dependent. The approach could have major implication for detection and monitoring of pancreatic and other cancers.

Commentary on Kimbrough et al.

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 post injection, mice were imaged by employing of 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, since 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 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, since clonal selection leads cell populations to overcome the therapy by growth of sub-populations that do not express sensitizing levels of the biomarker in question. Cytotoxic therapies, whilst 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 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 pre-invasive tumor to a malignant invasive carcinoma. Although the acidity also varies within and among tumors, the general variation favors therapy, since 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, since 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 biological 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, so very precise tuning of chemical properties would be needed in a targeting agent. But, an important to outline that the pH is lowest at the surfaces of cells compared to the bulk extracellular pH and increases with distance from cellular membrane, and becoming normal in the vicinity of blood vessels . So, 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 capable to undergo 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 (Figure 1). pHLIP peptides possess dual delivery capabilities, making use of the energy of folding to translocate polar cargo molecules across phospholipid bilayer of 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. Since pHLIPs are in 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 membrane and release of energy. Depending on pHLIP sequence, protonatable residues could be differently located on membrane surface, which directly affects the rate of the protonation events at various pHs, and thus pK of peptides insertion into the membrane. 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 pancreatic ductal adenocarcinoma in human xenografts as well as PDAC and PanIN lesions in genetically engineered mouse models (12).

Kimbrough and colleagues also employed pHLIP variant 7 for the targeting of PDAC and demonstrated that precise mapping of tumors could be achieved by optoacoustic imaging. Pancreatic ductal adenocarcinoma 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 micro-metastatic 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 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 outcome of therapy for tumors of various origins, especially highly aggressive and metastatic ones.
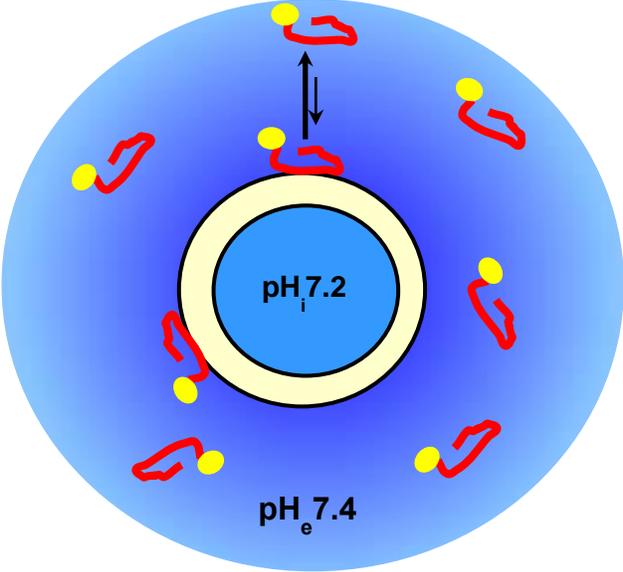
Figure Legend
Schematic presentation of pHLIP peptide (red) conjugated with fluorescent dye (yellow) interaction with plasma membrane of a normal cell in healthy tissue (A) and a cancer cell in tumor (B). The extracellular pH in healthy tissue is around pH7.4. On the other hand, pH gradient exists near 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 cell membrane in healthy tissue. However, at low extracellular pH in tumors, pHLIPs insert into 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 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.

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A   **Normal cell in healthy tissue**

pH$_i$7.2

pH$_e$7.4

B   **Cancer cell in tumor**

pH$_i$7.2

pH$_e$6.2–7.0