Published in final edited form as:
Gastroenterology. 2010 June ; 138(7): 2553–2554. doi:10.1053/j.gastro.2010.04.040.

Switching On the Light to See More Disease

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Summary

In this paper, Urano et al. demonstrate a new class of molecular probe based on a fluorescent small molecule that becomes 'activated' after becoming internalized into cells for use in performing *in vivo* imaging. This probe is developed from an aniline moiety that reacts with protons to produce light-induced electron transfer in a boron-dipyrromethene (BODIPY) fluorophore. Spontaneous relaxation of delocalized electrons to lower energy levels results in fluorescence emission at wavelengths >530 nm, a regime that can be distinguished from autofluorescence background in tissue. This small molecule produces undetectable fluorescence at a physiological pH of 7.4. The probes are 'switched on' inside the cell when they encounter an abrupt reduction in pH after being taken up by lysosomes. This novel imaging agent is used with a targeting agent, such as Trastuzumab, an antibody to HER2, a growth factor receptor that is over expressed on the cell surface of many important cancers including breast, esophagus, and lung. Targeting occurs when the Fab component of the probe binds to the HER2 receptor and initiates dimerization. The probe-receptor complex then becomes phosphorylated, internalized by endocytosis, and scavenged by lysosomes. The reduction in pH to a value between 5 and 6 results in the probe releasing an intense fluorescence signal. Furthermore, only live cancer cells are visualized because the low pH in lysosomes requires active proton pumps to maintain an acidic environment. This novel probe is shown to have high specificity for NIH3T3 cells and lung tumors that over express HER2. In addition, activation of the probe after it reaches inside the cell rather than when it is in the extra-cellular space results in a very high target-to-background ratio. This targeted imaging concept can be generalized to the detection of other over expressed cell surface receptors specific to cancer that results in cellular internalization.

Comment

Molecular imaging represents a new frontier in medicine, and this shift in paradigm is making rapid inroads into the field of gastroenterology (1). Currently, diagnostic decisions are made during endoscopy based on observing structural changes and identifying anatomical landmarks. Instead, molecular imaging provides information about diseased tissue based on visualizing functional properties and protein expression, including important mechanisms that drive the progression of disease, such as the upregulation of growth factors, activity of proteolytic enzymes, and expression of cell adhesion molecules. Although advances in technology have greatly improved the imaging performance of endoscopes over the past decade, the resolution of these instruments are limited by the optics and will never

be sufficient to directly observe the molecular changes associated with a number of diseases. Thus, visualizing the molecular expression pattern of cells and tissues requires the use of exogenous probes. Most probes used in molecular imaging are attached to a label that is 'always on,' and any non-specific binding can result in a reduction in image contrast. In this paper, Urano et al describes the development of a novel class of molecular probe that binds to cancer specific receptors, enters the cell, and becomes activated by a reduction in pH. In other words, this probe has a label that is 'off' in the tissue microenvironment and becomes switched 'on' only after entering a diseased cell to reveal its location. The release of fluorescence produces a high optical contrast for overcoming tissue background. This novel probe demonstrates several properties that are important for clinical use: 1) non-fluorescent at physiological pH, the condition of the extracellular space; 2) intensely fluorescent at low pH, the environment inside cell lysosomes; 3) ease of conjugation to targeting agents, means for achieving specific interactions with diseased cells; and 4) tunable to different acid dissociation constants (pK_a), allowing for the use of multiple 'switches.'

The first 'smart' probe was used to identify dysplastic polyps in $APC^{min/+}$ mice, and was developed based on the concept of dequenching (2). These near-infrared probes produce no fluorescence, being silenced by resonance energy transfer, until they encounter the presence of proteases, such as cathepsin and matrix metalloproteinase, that cleave a lysine bond within the probe. While these proteolytic enzymes play an important role in cell proliferation, invasion, apoptosis, angiogenesis, and metastasis, they define a limited set of molecular targets. On the other hand, the novel small molecule probe presented in this paper uses a mechanism for activation based on affinity binding and internalization. As a result, over expressed cell surface molecules can be targeted in addition to upregulated enzymes. Targeting can be achieved using a number of different agents, including antibodies (demonstrated in this paper), antibody fragments, aptamers, and peptides (3). Moreover, this mechanism has been exploited to identify targets for drug therapy in addition to performing diagnostics. The biologic relevance of HER2 as a therapeutic target has already been well established for breast cancer. New targeted therapies are being developed for colorectal cancer, including Cetuximab and Panitumumab against EGFR and Bevacizumab against VEGF. Visualization of these over expressed targets can be used to select the appropriate therapy for each individual patient to achieve the best outcome. Because disease develops from genetic changes that are unique to each person, the specific molecular mechanisms that are predominant require customized therapies for improved efficacy. Molecular imaging represents an exciting direction that requires the continued development of new and improved probes that provide more information about the biology of disease, and here is a promising new one.

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