Molecular Beacons Illuminate Subcellular Events

Robert O. Bonow, MD

Building on decades of technological advances and imaging experience, during which noninvasive imaging of cardiac structure and function, myocardial perfusion and viability, and noncoronary vascular pathology have become intimately entwined in routine clinical practice, advanced imaging techniques are now poised to deliver noninvasive coronary arteriograms and to delineate the coronary artery wall in exquisite detail. At the same time that these highly promising methods for anatomic imaging at the macroscopic level are creating great excitement among physicians, their patients, and the public, a quieter imaging revolution is underway with steady progress in detecting and tracking fundamental biological processes at the cellular and subcellular levels. In the era of genomic research, molecular biology, and stem cell therapies, these methods have great potential to accelerate understanding of basic pathophysiological processes in animals and humans and to develop new tools for early diagnosis and drug development. Cardiovascular molecular imaging is taking hold.

The prerequisite for an effective agent for imaging at the molecular level is molecular specificity for the intended target, such as a receptor, an enzyme, or a gene product. Targeted image agents are produced by identifying an antibody or peptide with affinity to the target, which is then attached to an isotope for nuclear imaging, an acoustic microbubble or liposome for ultrasonic imaging, a magnetic compound for MRI, or a bioluminescent probe or fluorochrome for optical imaging. Radionuclide antibodies were among the first such targeted agents. Targeted antibody-based agents provide high specificity for the target and are relatively simple to construct, but a common limitation is a high level of background noise related to unbound fractions or, in some cases, nonspecific binding.

A more exciting group of molecular probes is activatable imaging agents that undergo a physicochemical change and become detectable only after specific molecular interaction with the target. These “smart” agents have also been termed molecular beacons or sensors. Target specificity is high with little background noise, as the beacon becomes activated and is detectable only when the reaction of interest has occurred. Activatable imaging agents have been developed for both magnetic compounds and fluorescent compounds. In the latter category, novel activatable near-infrared (NIR) fluorochromes have been engineered for detecting, localizing, and quantifying specific protease activity.

These beacons possess unique quenching-dequenching properties such that they are optically inactive in their native quenched state and become highly fluorescent when dequenching occurs as the result of enzymatic cleavage of specific peptide sequences by the protease, with signal amplification of up to 1000-fold. In contrast, targeted agents have no amplification, and MRI-based activatable sensors have only 2- to 10-fold amplification. The NIR spectrum has advantages over visible light and infrared light for penetrating deeper tissues (more than a few millimeters) because hemoglobin, the principal absorber of visible light, and water and lipids, the principal absorbers of infrared light, have their lowest absorption in the NIR range of 650 to 900 nm. NIR also minimizes autofluorescence from nontarget tissues.

Capitalizing on these concepts, Chen et al designed and synthesized a novel NIR fluorescent (NIRF) probe to interrogate matrix metalloproteinase (MMP) activity in the myocardium after experimental myocardial infarction in mice, as reported in this issue of Circulation. The probe was de-

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signed with a peptide sequence that is recognized and cleaved by the gelatinases MMP2 and MMP9. Building on previous experience with other protease-specific NIRF beacons,8,9,13 the probe contains multiple fluorescent molecules in close proximity attached to the recognition peptide sequence, which in the intact state are effectively quenched by fluorescent resonance energy transfer. After proteolytic cleavage of the peptide sequence by the MMP, the molecule undergoes configurational change, releasing the fluorescent molecules, and the beacon “lights up” with 200-fold signal amplification.

Chen et al studied the application of this activatable NIRF beacon in mice at varying time intervals up to 4 weeks after myocardial infarction induced by ligation of the left anterior coronary artery. After intravenous injection of the imaging agent and subsequent euthanasia, NIRF imaging of the myocardium correlated well with immunohistochemical staining for gelatinases. Increased NIRF activity was detected within 2 days, peaked at 1 to 2 weeks, and persisted at 4 weeks. The increase in MMP activity was confirmed by gelatinase zymography and quantitative real-time polymerase chain reaction analysis of MMP2 and MMP9 mRNA levels, which demonstrated peak MMP9 expression at 2 to 4 days and MMP2 expression at 1 to 2 weeks. Dual-label in vivo confocal microscopy showed colocalization of MMP activity with neutrophils on day 1, and flow cytometric analysis confirmed that the NIRF signal is associated with leukocytes in the infarct zone. These results confirm that myocardial MMP activity is increased after myocardial infarction, supporting previous work implicating MMPs in the postinfarction left ventricular remodeling process,14–16 and implicate neutrophil infiltration as the likely source of MMP activity.

More broadly, the results of the study by Chen et al12 also underscore the rapid, exciting technological breakthroughs that are occurring in molecular imaging on many fronts. The ability to measure myocardial protease activity with unique activatable NIRF probes may lead to further studies of pharmacological interventions to limit this activity or its downstream effects.8 NIRF probes also have the potential to interrogate protease activity in vivo, both spatially and temporally. To move to the next level requires the application of imaging techniques to evaluate NIRF activity in living animals and ultimately in humans. Fluorescent molecular tomography (FMT) represents an innovative new imaging method to provide 3-dimensional images of NIRF activity, which has been used thus far to image protease activity in atherosclerotic plaques and tumors in small animals,1,7–9,17 This technology has possible application in large animals and in humans because NIR can penetrate 7 to 14 cm, depending on the tissue characteristics,8 but such penetration requires highly sensitive photon detection systems. Coupling FMT with other advanced imaging modalities, such as CT or MRI, will provide accurate anatomic localization.8,9 Invasive optical imaging approaches with catheter-based systems such as optical coherence tomography or fiberoptic imaging systems18,19 to study protease activity in atherosclerotic plaques are an alternate pathway for development.

Radionuclide imaging techniques continue to have advantages for in vivo molecular imaging in large animals and humans because tracer amounts of target-specific isotopes can be imaged routinely in deep tissues with sufficient sensitivity by SPECT or PET. In concert with the investigations of MMP activity by NIRF probes, designer nuclear probes have been developed to investigate MMP activity in experimental myocardial infarction20 and atherosclerotic plaques,21 as well as other markers for plaque matrix and inflammation.3,22 Alternative approaches to measure inflammatory cell activity include targeted liposomes for intravascular ultrasonic imaging2 and superparamagnetic iron oxide nanoparticles, which undergo phagocytosis by activated macrophages, for MRI.7 Both of these techniques have their individual strengths and shortcomings, but in general they serve as targeted molecular probes and do not have the particular advantages of activatable NIRF probes, principally signal amplification, as noted previously.

Molecular imaging is advancing in many important directions, but stem cell biology represents an area in which subcellular imaging will play an absolutely essential role. Stem cells can be transfected with optical or nuclear reporter genes or labeled with nanoparticles23,24 that can be imaged noninvasively in the heart. Along with existing methods for evaluating cardiac structure, function, and tissue perfusion, these molecular probes will provide critical information regarding the success or failure of stem cell strategies to regenerate myocardial tissue, enhance angiogenesis, or develop biological pacemakers.

As molecular imaging evolves during the next several years, advances in subcellular imaging will undoubtedly be coupled tightly to the ongoing advances in noninvasive imaging at the macroscopic, whole-organ level. Systems to detect bioluminescent, fluorescent, and nuclear probes will never achieve the spatial resolution afforded by MRI and CT. It is conceivable that NIRF or nuclear probes will signal the presence and magnitude of a molecular event and that a coincident CT or MR image will identify the precise location of that event.8,9 The next generation of SPECT and PET scanners will merge nuclear technology with CT technology with hybrid CT/PET and CT/SPECT systems.25 It is likely that similar CT/FMT or MRI/FMT systems will also emerge in the near future to capture the signal from NIRF beacons.

References


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