Molecular imaging is a unique research discipline with the potential to detect disease in pre-clinical and early stages. It integrates fields of clinical medicine, cell biology, and signal transduction for appropriate selection of target to be imaged, and synthetic chemistry and imaging sciences for characterization of the pathology. In contrast to traditional diagnostic techniques, molecular imaging employs noninvasive, quantitative, and repetitively feasible strategy of imaging targeted biological processes at both cellular and subcellular levels within a living organism (1).

Due to recent advances in nanotechnology and engineering innovations, molecular imaging has undergone profound change during its rather short development period as a scientific discipline. By weaving medicine and computer technology together, molecular imaging has uncovered simplicity behind the complexity of diseases. It has offered quantitative assessment of pathophysiology, altered metabolic states, subcellular processes, receptor modifications, and message or gene expression in various disorders. To effectively develop the concept of molecular imaging, it has become essential that we demonstrate the feasibility of imaging in small transgenic animal models. This has stimulated the development of advanced small animal imaging technology including microscale positron emission tomography, single-photon emission computed tomography, computed tomography, magnetic resonance imaging, high-frequency ultrasound, and optical fluorochrome or bioluminescence imaging. It is expected that fusion technology involving computed tomography, magnetic resonance imaging, or ultrasound imaging combined with nuclear or optical imaging would not only provide both morphological and functional information, but also better localization of hitherto difficult foci of tracer uptake.

It is exciting that the National Cancer Institute has identified molecular imaging as 1 of the 6 most rewarding opportunities for development (2), and a major thrust has been placed on molecular imaging in the newly formed National Institute of Biomedical Imaging and Biomedical Engineering. Although the National Heart, Lung, and Blood Institute has yet to fully embrace this initiative, the field of cardiovascular molecular imaging has slowly but surely progressed. In addition to addressing a diagnostic query, we strongly believe that targeting of key steps in cellular or subcellular biologic processes further clarifies critical pathogenetic pathways. The pathophysiology of a disease process and development of targeted imaging are interdependent and complementary. Review of the historical evolution of any molecular imaging strategy should substantiate our claim.

Let's consider the imaging of apoptosis in myocardium. It becomes amply clear that targeting of a cell membrane phospholipid, phosphatidyl serine (PS, a hallmark of apoptosis), not only allowed development of a diagnostic tool, but has also led to better understanding of reversibility of...
Apoptotic process, in turn expanding diagnostic applications and proposing novel intervention strategies.

Apoptosis, a programmed cell death process, constitutes an integral part of the pathology associated with ischemic or inflammatory state of the myocardium (3); it is believed to precede the necrotic change. It has been proposed that noninvasive imaging of apoptosis may allow early identification of myocardial damage and since damage follows an ordained cascade, it may be amenable to recovery. The apoptosis-inducing stimuli usually converge to release various mitochondrial proteins into a cytoplasmic compartment, which initiate a chain reaction to activate highly specific proteolytic enzymes, referred to as terminal caspases, such as caspase-3 (4). Activation of caspase-3 is linked to the loss of asymmetry of phospholipid distribution between the inner and outer cell membrane layers, and has been explored as one method of apoptosis imaging. PS, which is normally restricted to the inner membrane tablet is exteriorized to the outer cell membrane layer, and has been targeted by appropriately labeled annexin A5 (AA5) for in vitro and in vivo imaging; AA5 is a normally circulating protein and has a nanomolar affinity for PS. Elegant cell culture experiments had paved the way for initial imaging of apoptotic cells, and a radiolabeled AA5 has been used clinically for molecular imaging of apoptosis in myocardial infarction and transplant rejection (5–7). Experimental evidence confirmed AA5 uptake and biochemical activation of caspase-3, but was not verified by histologic evidence of either apoptosis or necrosis. Ultracentrifugal separation of myocardial homogenates surprisingly recovered radiolabeled AA5 from the cytoplasmic compartment suggesting the internalization of PS-bound AA5. Subsequently, a series of experiments revealed that PS exteriorization occur within 5 min of ischemic insult, which is preventable by preadministration of caspase inhibitors (13). In the absence of caspase inhibitors, PS remains expressed on the outer cell membrane up to at least 6 h after resolution of brief ischemia, potentially facilitating after-the-fact imaging of ischemia. The PS exteriorization was found to be a continuous process until caspase-3 activation spontaneously resolved. The PS-bound AA5 trimerized between 10 to 20 min and invaginated a membrane patch into the cytoplasm as a vesicle and removed the PS from surface; persistent expression of PS would have constituted an “eat-me-signal” for phagocytosis of the doomed cell. It becomes much clearer from the aforementioned description that whereas bench-side cues are important for development of molecular imaging, the observations made by molecular imaging equally and substantially contribute to a better understanding of the disease process.

The story goes a bit further into apoptosis imaging of the myocardium. A slow and smoldering apoptosis occurs in remodeling myocardium and contributes to an inexorable decline in ventricular function in heart failure (HF). Cytokine and/or oxidative stress results in release of cytochrome c from the mitochondria into cytoplasmic compartment, and consequent activation of pro-apoptotic caspase-3 results in myofilament proteolysis and DNA fragmentation (14). However, simultaneous upregulation of antiapoptotic factors, such as BC12- and XIAP-like proteins, and loss of DNA
fragmentation factors prevent completion of apoptotic process (apoptosis *interruptus*) (4,15). The amount of activated caspase-3 is determined by the balance of antiapoptotic and proapoptotic factors. Because caspase-3 is activated in HF, it was proposed that the attendant PS externalization should be amenable to AA5 imaging in HF (1). The AA5 uptake was observed in up to 45% of patients with worsening HF, who went on to demonstrate a 10% decrease in their ejection fraction after 1-year follow up. On the other hand, the remaining patients with negative scans improved LV function by 7% in the follow-up. The results suggested that fewer the endogenous antiapoptotic factors, the greater the residual caspase-3, the more PS externalization, the higher the necessity for supportive therapy. It is evident from the foregoing discussion that AA5 imaging has contributed immensely to our understanding of the intricacies of heart muscle cell damage in various cardiovascular diseases (Table 1).

The convergence of disciplines in medicine, molecular and cellular biology, and computer technology will likely translate into development of better diagnostic and management strategies. It is important that we strive to understand the pathogenesis of various cardiovascular disorders. Identification of the worthy targets which are prominently overexpressed in the diseased tissue would provide a high target-to-background contrast for hot-spot imaging. Alternatively, identification of moieties which are conspicuously absent from the target tissue would allow a cold-spot pattern of imaging. Molecular imaging is not limited by imaging modality. Once a target and targeting agent is identified, the imaging can be performed by conjugation of the imaging probe with appropriate reporting tracer and the target should become amenable to respective modality of imaging. In this issue of *JACC: Cardiovascular Imaging*, one of the papers proposes the imaging of angiotensin-II type 1 receptors, which is likely to uncover susceptibility to the development of adverse myocardial remodeling and HF (17). The paper (17) and the editorial comment (18) are linked to CVN interviews that highlight the role that molecular imaging will play in clinical medicine. It is mandatory that an effort be made to express the conceptual premises and practical execution in a simplified manner so as to stimulate close collaboration among the clinicians, biologists, and imagers alike. Molecular imaging teaches us that targets are a plenty in health and disease, each with a story to tell. Imaging and imagers are the future story tellers, the Rosetta stone of pathophysiology and advanced therapeutics.

Happy targeting, folks!

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**Table 1. Evolution of Molecular Imaging of Apoptosis in Myocardium**

<table>
<thead>
<tr>
<th>Disease Process</th>
<th>Pathogenetic Basis of Imaging</th>
<th>Imaging Results</th>
<th>Imaging Leading to Pathogenetic Clues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>Apoptosis and necrosis in the areas of myocardial damage (1)</td>
<td>AA5 uptake observed in the areas corresponding to perfusion deficit (5)</td>
<td>Caspase inhibitors resolve AA5 uptake (8,10), decrease both apoptosis and necrosis (9), establish apoptosis-necrosis continuum, extend window for intervention (8)</td>
</tr>
<tr>
<td>Myocardial ischemia</td>
<td>Apoptosis precedes necrosis (1)</td>
<td>AA5 uptake observed in ischemic area not associated with histologically-verified apoptosis or necrosis; AAS recovered from cytoplasm (11)</td>
<td>Transient PS expression and AA5 uptake, AAS removes PS from membrane by invagination as a protective phenomenon (13), caspase inhibition hastens recovery</td>
</tr>
<tr>
<td>Heart failure</td>
<td>Incomplete apoptosis contributes to ventricular dysfunction (3,4,15)</td>
<td>In worsening HF patients AA5 uptake is associated with poor outcome (16)</td>
<td>Simultaneous upregulation of pro- and anti-apoptotic factors establish the basis of incomplete apoptosis in heart failure and suggest novel avenue for reverse remodeling (4,16)</td>
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</tbody>
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