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Radiolabeled Duramycin: Promising Translational Imaging of Myocardial Apoptosis

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Ischemic heart disease is the leading cause of death globally that accounts for approximately 7.4 million deaths every year (1). It kills cardiomyocytes due to the limited blood supply from obstruction of coronary arteries. Although reperfusion therapy is routinely used to restore the blood supply for ischemic myocardium, this treatment may paradoxically worsen cardiomyocyte death through reperfusion-induced inflammation and oxidative damage (2). When the ischemia-reperfusion injury (IRI) occurs, the loss of cardiomyocytes is usually initiated by apoptosis, a programmed death of cells associated with various cardiovascular diseases (3). Studies showed that inhibiting apoptosis could reduce reperfusion injury and attenuate the following ventricular remodeling and heart failure (4,5). To achieve better outcomes, therapeutic interventions need to be performed in a timely manner with the assistance of early apoptosis detection. To this end, molecular imaging has played an indispensable role in detecting myocardial apoptosis *in vivo* (6). In addition, molecular imaging also allows for noninvasive and systematic assessments on these therapeutic interventions.

Molecular imaging of apoptosis commonly uses radiolabeling to target molecular markers associated with apoptosis process, including externalized phospholipid, activated caspases, and dissipated mitochondrial transmembrane potential ($\Delta\psi_m$) (Figure 1) (7–12). After administrating radiotracers, radionuclide imaging is used to detect the radiolabeled apoptosis cells *in vivo*. For the detection of myocardial apoptosis, the typical approach combines single photon emission computed tomography (SPECT) imaging with Technetium-99m-labeled annexin V (^{99m}Tc-Annexin-V), which has been proven to detect IRI and acute cardiac allograft rejection in several clinical trials (13,14). It uses annexin V to bind to the

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None.

externalized phosphatidylserine (PS) on the outer leaflet of apoptotic cell membrane, one of the phospholipid alterations of cell membrane associated with apoptosis. However, as a large molecule with positive charges, ^{99m}Tc-Annexin-V inflicts significant radiation burden to non-target organs, especially to the kidney (14,15). Recently, minimizing exposure to radiation burden has received increasing attention in cardiovascular imaging (16,17), particularly for serial monitoring of cardiac therapies. In the last decade, the problem of significant radiation burden has in part slowed down the clinical applications of ^{99m}Tc-Annexin-V. Our recent studies indicated that radiation burden from cardiac radionuclide imaging could increase the DNA damage and gene activations for apoptosis and DNA repair (18,19). This shows that to facilitate apoptosis imaging for cardiovascular diseases, a more advanced imaging approach with sensitive detection of myocardial apoptosis but reduced radiation burden is needed.

To provide an alternative approach, Kawai et al. validated the feasibility of using radiolabeled Duramycin to detect myocardial apoptosis post-IRI with effective target uptake but reduced radiation burden for non-target organs (20). ^{99m}Tc-Duramycin was developed to bind to the externalized phosphatidylethanolamine (PE), another phospholipid maker on apoptotic cell membrane (21). Similar to PS, PE is a rich phospholipid in the inner leaflet of the cell membrane but will externalize onto the outer cell surface during apoptosis. By employing ^{99m}Tc-Duramycin to target PE, SPECT can detect the apoptotic cardiomyocytes post-IRI (22). In this study, Kawai et al. made several important findings on imaging myocardial apoptosis with ^{99m}Tc-Duramycin. First, using IRI animal models, they demonstrated that similar to ^{99m}Tc-Annexin-V, ^{99m}Tc-Duramycin was also an effective radiotracer to target myocardial apoptosis. *In vivo* microSPECT imaging showed that targeting PE with ^{99m}Tc-Duramycin detected similar myocardial apoptosis compared to targeting PS with ^{99m}Tc-Annexin-V. Significantly higher uptake of both tracers was found in the infarct region compared to the remote zone, demonstrating the specific uptake of ^{99m}Tc-Duramycin for the myocardium with elevated apoptosis. Additionally, simultaneous administration of fluorescent Duramycin and Annexin V showed that both tracers co-localized in the same infarcted regions. Second, they found an important advantage of ^{99m}Tc-Duramycin over ^{99m}Tc-Annexin-V in that ^{99m}Tc-Duramycin led to significantly lower radiation burden to the kidney (Duramycin: 0.358±0.210 %ID/g versus Annexin V: 1.58±0.316 %ID/g, $P<0.001$). Moreover, as Duramycin was a small 19-amino-acid peptide that could be rapidly cleared from the kidney, its overall off-target radiation was also less than that of ^{99m}Tc-Annexin-V. Lastly, besides detecting IRI, ^{99m}Tc-Duramycin could also detect the decreased apoptosis in minocycline-treated acute myocardial infarction, making it possible to use ^{99m}Tc-Duramycin for serial monitoring of therapeutic interventions for IRI.

The aforementioned advantages of ^{99m}Tc-Duramycin highlight its translational potential for clinical applications. Compared to the well-established apoptosis imaging of ^{99m}Tc-Annexin-V, ^{99m}Tc-Duramycin imaging is equally effective at detecting myocardial apoptosis but with less radiation burden, which overcomes an important clinical limitation of current apoptosis imaging. Moreover, as an antibiotic molecule, Duramycin has been used safely in humans, thus could be translated rapidly into clinics. In addition, its favorable dynamic range for detecting apoptosis allows ^{99m}Tc-Duramycin imaging to monitor therapeutic interventions by tracking the diminishing levels of apoptosis after anti-apoptotic therapies.

However, before its clinical translation, ^{99m}Tc-Duramycin still needs more comprehensive validation on long-term safety and effective detection of myocardial apoptosis in patients. Future efforts may also involve exploring the clinical application of ^{99m}Tc-Duramycin imaging in other cardiovascular diseases, including heart failure, atherosclerosis, and cardiac allograft rejection.

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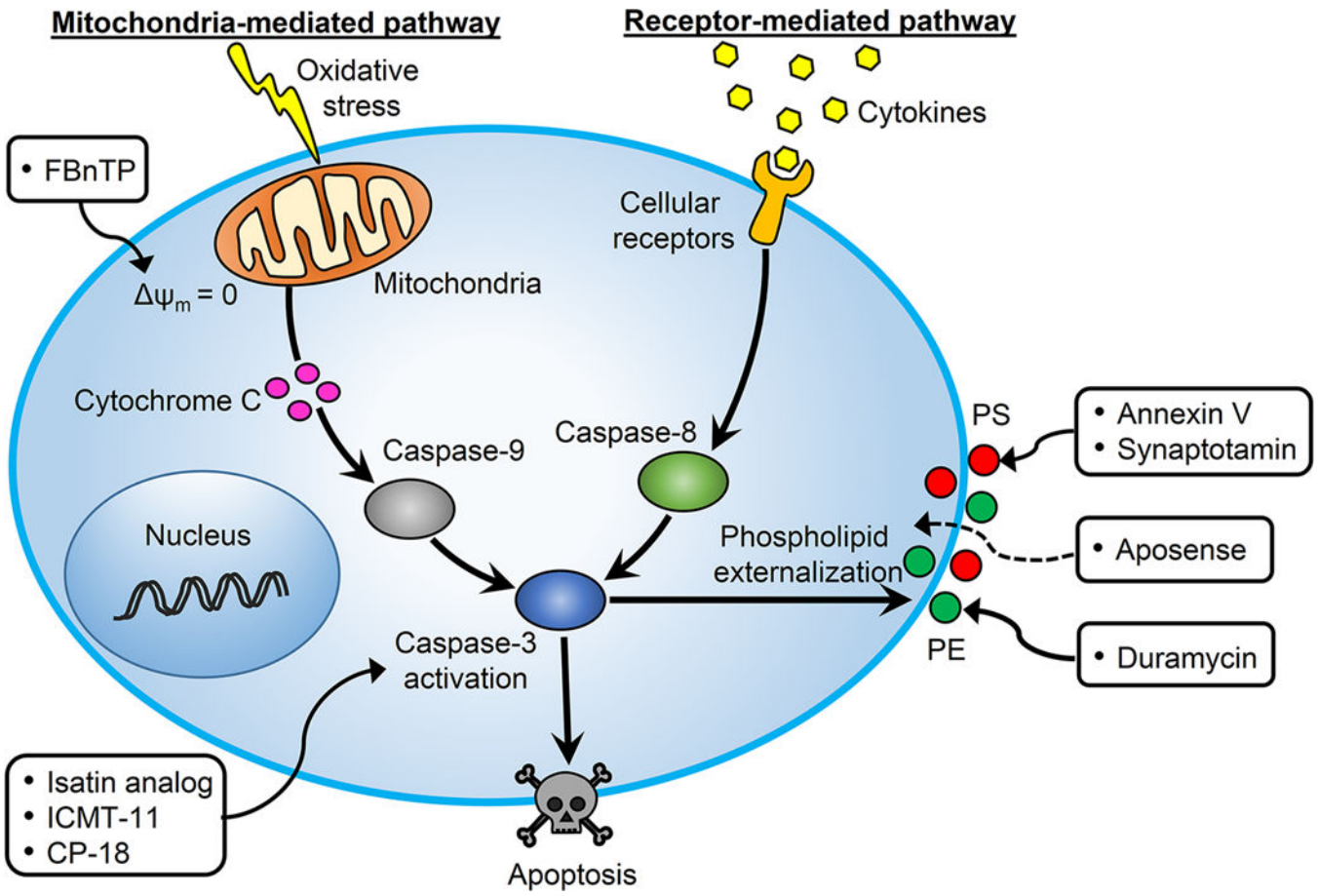

Figure 1. Central pathways and imaging targets for apoptosis.

Both extrinsic pathway (cell-surface receptors) and intrinsic pathway (mitochondria) activate caspase-3 to finally result in apoptosis. The externalized phospholipid (i.e., PS and PE), activated caspase-3, and dissipated $\Delta\psi_m$ can be targeted by various radiotracers for noninvasive imaging of apoptosis. PS: phosphatidylserine; PE: phosphatidylethanolamine; $\Delta\psi_m$: mitochondrial transmembrane potential.