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EDITORIAL COMMENT

Improving Left Ventricular Myocardial Function After Myocardial Infarction

Is it in the BAG?*

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Each year in the in the United States, ~1.5 million patients suffer acute myocardial infarction (MI), with 500,000 consequent deaths. Best outcomes occur when patients receive fibrinolysis infusion or percutaneous coronary interventions to restore blood flow through occluded vessels within 30 to 90 min of admission. Later interventions to preserve cardiac function, limit progressive cardiac remodeling (dilation, fibrosis), and arrhythmias deploy a panoply of therapeutics (beta-blockers, angiotensin-targeted drugs, statins, aldosterone-antagonists, antiarrhythmics). Despite this armamentarium, rehospitalization because of worsening cardiac dysfunction occurs in almost one-half of survivors within the first year. Thus, an essential goal remains: the identification of novel pathways that underlie progressive deterioration of cardiac function and that provide innovative opportunities for therapeutic intervention.

Apoptosis and autophagy are cell death processes that accompany acute cardiac MI and late remodeling (1,2). For decades, researchers have studied the mechanism by which Bcl-2 proteins regulate apoptosis (3). The search for proteins that interact with Bcl-2 led to the discovery of the BAG family of proteins (Bcl-1-associated athanogenes). At least 6 family members are recognized with a high degree of phylogenetic conservation in the BAG domain (an ~70 amino acid region that binds to the ATPase binding domain of Hsc/Hsp70). Hsc/Hsp70 proteins are ubiquitously expressed and central components of the cellular system for managing unfolded, misfolded, or aggregated proteins (4,5). In particular, BAG3 is a key member of the Hsp70 complex that regulates proteosomal and autophagic processes (5). BAG3 is expressed at high levels in cardiac and skeletal muscle and in several cancers (6,7). Elevated expression of BAG3 is antiapoptotic and associated with resistance to cancer chemotherapeutics (7). BAG3 has multiple protein-interacting domains allowing interaction with small heat shock proteins and proteins central to many signaling pathways that contain PDZ and SH3 domains (6). In HeLa cells, a BAG3-protein interactome of more than 350 proteins of diverse cellular processes has been identified (8), reflecting a broad participation in cell function.

BAG3 was linked to cardiac dysfunction when it was recognized that BAG3-deficient mice developed a severe myopathy and early death, that variants in the BAG3 gene were associated with childhood muscular dystrophy and dilated cardiomyopathy, and that humans with idiopathic dilated cardiomyopathy show reduced cardiac expression of BAG3 protein (6,9–12). Among its myriad protein interactions, BAG3 maintains sarcomeric structure through interactions with actin capping protein (CapZβ1) and Hsc70 (13). BAG3 also interacts with β1 adrenergic receptors and Ca^{2+} channels, linking BAG3 to striated muscle (dys)function (14). These observations provide the rationale for BAG3 as a therapeutic target in cardiac dysfunction.

In this issue of *JACC: Basic to Translational Science*, Knezevic et al. (15) sought to test the hypothesis that gene delivery of BAG3 to the hearts of mice with left ventricular dysfunction secondary to MI could enhance cardiac performance. Eight

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weeks after left coronary artery ligation (sham surgery), mice received systemic delivery of rAAV-9 encoding either green fluorescent protein (AAV-GFP, control) or BAG3 (AAV-BAG3). Mice were then followed for 3 weeks. At the time of tissue harvest, MI-BAG3 mice showed about twice the level of cardiac BAG3 when compared with MI-GFP mice reflecting a near normalization of BAG3 expression. Echocardiography demonstrated that, as anticipated in this model, MI mice that received AAV-GFP (MI-GFP) showed significantly larger left ventricular (LV) chamber dimensions, volumes and mass, thicker posterior walls, reduced systolic function (ejection fraction, fractional shortening), and reduced stroke volume relative to Sham-GFP mice.

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Relative to MI-GFP mice, MI mice that received AAV-BAG3 (MI-BAG3) showed decreased wall thickness, improved systolic functional measures (ejection fraction, fractional shortening), and an increased stroke volume. Analysis of cardiomyocytes isolated 3 weeks after AAV-BAG3 injection revealed improved (relative to MI-GFP cardiomyocytes) isoproterenol-stimulated maximal cell shortening and kinetics of contraction/relengthening as well as improved maximal calcium transient amplitudes. The improvements in isoproterenol stimulated calcium transients and contractile kinetics reinforces the prior observation from this group that BAG3 couples the β1 adrenergic receptor and L-type calcium channels. The authors cautiously suggest that the restoration of BAG3 expression can improve cardiac function; this serves as a proof of concept for use of BAG3 as a potential heart failure therapeutic.

Because this appears to be the first utilization of a gene-therapy vector to achieve BAG3 overexpression in any disease setting, it is a reassuring sign that no deaths were observed in any mice receiving AAV-BAG3. In addition, the improvements of systolic function and calcium handling are encouraging. However, several limitations should also be considered. For example, mice were not followed for more than 3 weeks post-BAG3 gene delivery, presenting a narrow window for detection of adverse events. Although arrhythmic events may be unlikely to be exacerbated by BAG3 delivery because of normalization of calcium transients in isolated cardiomyocytes, arrhythmias arise from structural and electrical heterogeneities in the cardiac substrate, and no measures of arrhythmias (spontaneous or provoked) in intact hearts were reported. The measured endpoints do not indicate improvements in post-MI cardiac remodeling (MI-BAG3 hearts showed persistently enlarged LV dimension and masses). Changes in fibrosis were also unreported. The lack of significant changes in endpoints associated with heart failure (no assessment of pulmonary edema, no change in BNP expression) makes it unclear whether heart failure persists, but with an altered phenotype. Furthermore, although the authors have focused on some of the many important roles of BAG3 (improvement of calcium handling, particularly in response to beta-adrenergic stimulation), there is no assessment of apoptosis or autophagy in a system in which these are important processes.

Although the authors will likely assess such endpoints in future models, there are obstacles ahead. BAG3 expression or overexpression has been linked to the resistance of many cancers to therapeutic agents (7). Patients with ischemic heart failure have a doubled risk of cancer (16); what's the risk if one further enhances BAG3 expression, perhaps in off-target organs? Approaches to restrict gene delivery and exogenous BAG3 expression to the myocardium would be pivotal. Although gene therapy vectors are making their way into clinical trials, other approaches could alter endogenous BAG3 expression with low molecular weight compounds, assuming an enhanced cancer risk is not evident. For example, basic fibroblast growth factor (FGF-2) is reported to increase BAG3 expression in neuroblastoma cells (17). At least 1 trial, which delivered intracoronary FGF-2 protein to patients with severe ischemic heart disease in an attempt to increase cardiac angiogenesis, showed some improvements in LV wall thickening and reduced ischemic area (18). It is unknown whether these results arose from induced cardiac BAG3 expression.

In summary, the gene therapy-directed overexpression of BAG3 improves cardiac function in a murine model of chronic ischemic injury in the immediate post-delivery interval, and shows promise as a therapeutic for post-MI improvement of cardiac function. The myriad processes through which BAG3 regulates multiple cellular functions, and the best way to harvest such potential for a post-MI therapeutic, does not (yet) place BAG3 gene therapy "in the bag."

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