Activation of the Homeostatic Intracellular Repair Response During Cardiac Surgery

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BACKGROUND: The homeostatic intracellular repair response (HIR2) is an endogenous beneficial pathway that eliminates damaged mitochondria and dysfunctional proteins in response to stress. The underlying mechanism is adaptive autophagy. The purpose of this study was to determine whether the HIR2 response is activated in the heart in patients undergoing cardiac surgery and to assess whether it is associated with the duration of ischemic arrest and predicted surgical outcomes.

STUDY DESIGN: Autophagy was assessed in 19 patients undergoing coronary artery bypass or valve surgery requiring cardiopulmonary bypass. Biopsies of the right atrial appendage obtained before initiation of cardiopulmonary bypass and after weaning from cardiopulmonary bypass were analyzed for autophagy by immunoblotting for LC3, Beclin-1, autophagy 5-12, and p62. Changes in p62, a marker of autophagic flux, were correlated with duration of ischemia and with the mortality/morbidity risk scores obtained from the Society of Thoracic Surgeons Adult Cardiac Surgery Database (version 2.73).

RESULTS: Heart surgery was associated with a robust increase in autophagic flux indicated by depletion of LC3-I, LC3-II, Beclin-1, and autophagy 5-12; the magnitude of change for each of these factors correlated significantly with changes in the flux marker p62. In addition, changes in p62 correlated directly with cross-clamp time and inversely with the mortality and morbidity risk scores.

CONCLUSIONS: These findings are consistent with preclinical studies indicating that HIR2 is cardioprotective and reveal that it is activated in patients in response to myocardial ischemic stress. Strategies designed to amplify HIR2 during conditions of cardiac stress might have a therapeutic use and represent an entirely new approach to myocardial protection in patients undergoing heart surgery. (J Am Coll Surg 2013;216:719–729. © 2013 by the American College of Surgeons)

Heart surgery with cardioplegic arrest and cardiopulmonary bypass (CPB) is associated with postoperative complications resulting from myocardial injury. This is due in part to ischemia/reperfusion injury secondary to inadequate intraoperative myocardial protection manifest as stunning and necrosis.[1-3] Myocardial stunning (reversible post-ischemic myocardial dysfunction) presents as low cardiac output and lasts hours to days after surgery. In patients with limited functional reserve, stunning can lead to multi-organ failure and death. Similarly, some degree of myocardial necrosis occurs in up to 20% of patients undergoing heart surgery, with subsequent adverse short- and long-term consequences.[4-8] Because irreversible tissue injury involves cell death, previous efforts focused on preventing apoptosis and necrosis.[9] However, attention is now focused on mechanisms to salvage cells by amplifying their endogenous protective mechanisms.[10] One such mechanism is the homeostatic intracellular repair response (HIR2). The underlying process is adaptive autophagy, a lysosomal salvage pathway that eliminates damaged mitochondria and misfolded aggregated proteins in response to stress.[11] Adaptive autophagy is characterized by the formation of a cup-shaped pre-autophagosomal double-membrane

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Abbreviations and Acronyms

Atg = autophagy protein
CABG = coronary artery bypass grafting
CPB = cardiopulmonary bypass
HIR2 = homeostatic intracellular repair response
STS = Society of Thoracic Surgeons

structure that surrounds cytoplasmic material and closes to form the autophagosome (Fig. 1). The outer membrane of the autophagosome fuses with a lysosome to create a single membrane structure, the autophagolysosome. Here, the cargo is degraded and amino acids, free fatty acids, and sugars are exported to the cytosol for use as metabolic substrates.[12-14] This process involves multiple autophagy proteins (Atg). For example, activation of the class III phosphatidylinositide 3-kinase/Vps34 and Beclin1 (Atg6) complex results in the formation of an isolation membrane to which other autophagy proteins are recruited. The conjugate of Atg12-Atg5 is involved in the expansion of the isolation membrane. Another protein, p62, functions as an adaptor protein to link ubiquitin-rich mitochondria and protein aggregates to the forming autophagosome. Autophagic activity is reflected in changes in the abundance of proteins, such as Beclin-1, Atg5-12, and p62. Because p62 levels increase when autophagy is inhibited chronically, and decrease when autophagy is induced, p62 can be used as a marker to assess autophagic flux.[15-18] We chose key components of the autophagy pathway to monitor by Western blot, which previous studies had indicated were dynamically regulated or consumed during autophagy. Beclin-1 catalyzes modifications of lipids to form the initial autophagosomal membrane. Autophagy 12 is conjugated to Atg5; this complex is essential for extension of the membrane. LC3-I is conjugated to the membrane, converting it to LC3-II; LC3-II remains associated with the autophagosome until it is degraded in the lysosome. The adaptor protein p62 binds to organelles or aggregated proteins that are coated with ubiquitin, and facilitates their engulfment by the autophagosome.

Autophagy has been extensively investigated in cell culture and animal models subjected to stresses, such as starvation or ischemia/reperfusion.[14,19-21] To measure autophagy, it is necessary to obtain tissue for Western blot analysis, immunofluorescence microscopy, or electron microscopy. For that reason, few studies have examined autophagy in the human heart,[22,23] but there is evidence that HIR2 is active in animal models.[17,24-26] Given that inadequate myocardial protection remains a major clinical problem, the purpose of this study was to determine if HIR2 is activated in patients undergoing surgery, and whether the duration of ischemic arrest or patient risk factors affect the pathway.

METHODS

Study design

The study protocol was fully approved by the IRBs of Wayne State University School of Medicine and Oakwood Hospital Medical Center. All tissue procurements were done in compliance with the IRB-approved protocol. The transfer of human tissue between Oakwood Healthcare Inc. and the San Diego State University was governed by a Universal Biological Materials Transfer Agreement between the 2 institutions. Nineteen patients undergoing conventional heart surgery with CPB and intraoperative cardioplegia were studied. Inclusion

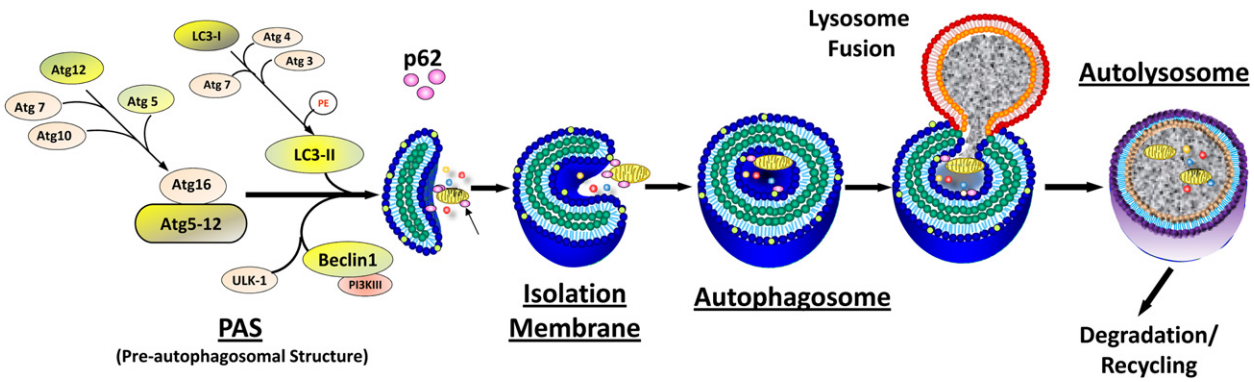

Figure 1. Autophagy machinery involved in homeostatic intracellular repair response. Initiation of Atg involves the participation of Atg proteins (Atg) involved in formation of the pre-autophagosomal structure and elongation of the isolation membrane. Autophagy 12 is covalently linked to Atg5 by a ubiquitin ligase system (Atg7, Atg10). Damaged mitochondria, protein aggregates, and other cytosolic components are flagged for engulfment by the adaptor protein p62. The autophagosome fuses with a lysosome and the cargo is degraded along with many of the autophagy proteins. Under conditions of rapid autophagic flux, autophagy proteins might be consumed before they can be replaced by new protein synthesis.