Phosphatase and tensin homolog-induced putative kinase 1 and Parkin in diabetic heart: Role of mitophagy

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ABSTRACT

Diabetes is an independent risk factor for cardiovascular morbidity and mortality. Diabetes-associated cardiac pathophysiology is recognized to be due to reasons including metabolic consequences on the myocardium. The heart is a highly energy-demanding tissue, with mitochondria supplying over 90% of adenosine triphosphate. The involvement of mitochondrial dysfunction in diabetes-related cardiac pathogenesis has been studied. Phosphatase and tensin homolog-induced putative kinase 1 (PINK1) and Parkin, initially identified to be associated with the pathogenesis of a familiar form of Parkinson's disease, have recently been recognized to play a critical role in mediating cardiomyocytes' adaption to stresses. Extensive studies have suggested PINK1 and Parkin as key regulators of mitophagy. In the present review article, we will first summarize the new findings on PINK1/Parkin acting in cardioprotection, and then discuss the potential role of PINK1/Parkin in diabetic heart by mediating mitophagy.

INTRODUCTION

The prevalence of diabetes mellitus has rapidly increased throughout the world. Diabetes mellitus increases the incidence of cardiovascular disease[1], which, in turn, has become the predominant cause of death in diabetic populations. Diabetes-associated cardiac complications are mainly a result of perturbed cholesterol, and vascular and platelet biology[2]. Modulation of mitochondrial function in cardiomycytes as a cause of diabetes-associated cardiac involvement is also suggested[2].

Phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) and Parkin, initially identified to be associated with the pathogenesis of Parkinson's disease, have recently been recognized as having a key role in mediating cardiomyocytes' adaption to stresses. Also, studies reported that the *pink1* transcription was suppressed in the skeletal muscle of type 2 diabetic patients[3], and protein levels of PINK1 and Parkin were reduced in the hearts of type 1 diabetic mice[4], suggesting a role of PINK1 and Parkin in diabetes. However, the involvement of PINK1/Parkin in the pathogenesis of diabetes-associated cardiac dysfunction has seldom been studied up to now.

One classical function carried by PINK1/Parkin is promoting mitochondrial elimination through autophagy, termed mitophagy. Indeed, autophagy is important for the turnover of organelles at low basal levels under normal conditions, and it is upregulated in response to stresses, such as starvation[5], infarction[6,7], ischemia/reperfusion[8] and pressure overload-induced heart failure[9]. Although baseline autophagy is necessary for maintaining cardiac homeostasis, the role of stress-induced autophagy is still controversial. Mitophagy is indispensable for mitochondrial quality control by removing aged and damaged mitochondria from the mitochondrial network. The mitophagic response to stresses has the potential to work as a double-edged sword, as enhanced levels of mitophagy can lead to loss of

mitochondria, which has a detrimental effect on maintaining cardiac function, while inefficient mitophagy leads to an accumulation of mitochondria with low membrane potential that produce less adenosine triphosphate (ATP) and more reactive oxygen species (ROS). Damaged mitochondria also undergo opening of permeability transition pores, which allows the release of proapoptotic factors and mitochondrial deoxyribonucleic acid (DNA) to induce apoptosis and inflammation.

In the present review, we first summarize the new findings on PINK1/Parkin acting in cardioprotection, and then discuss the potential role of PINK1/Parkin in the diabetic heart by mediating mitophagy.

PARKIN AND PINK1 MEDIATE SELECTIVE AUTOPHAGY OF DAMAGED MITOCHONDRIA

Loss of function mutation in genes encoding Parkin[10] and PINK1[11] was initially identified to be responsible for the pathogenesis of autosomal recessive juvenile forms of Parkinson's disease, in which mitochondrial dysfunction is widely reported. Recently, they have also been identified as regulators of mitophagy, and the mechanism has been briefly reviewed elsewhere[12,13].

Parkin is Recruited to Mitochondria by PINK1

PINK1 is a serine/threonine protein kinase targeted at mitochondria. In polarized mitochondria, PINK1 is imported and inserted into the inner membrane of mitochondria by the translocase of the outer membrane and translocase of the inner membrane 23 complex, depending on the voltage component of the mitochondrial inner membrane potential. Then PINK1 is processed to a smaller form by the mitochondrial rhomboid proteinase, presenilin-associated rhomboid-like protease, and released through an unclear mechanism to the intermembrane space or cytoplasm, where it is subsequently degraded by an MG132 sensitive protease[14,15]. On the dissipation of mitochondrial membrane potential, PINK1 accumulates as a full-length form on the mitochondrial outer membrane, recruiting Parkin from the cytoplasm to mitochondria[15,16].

Parkin Mediates Selective Mitophagy

Parkin is an E3 ubiquitin ligase that is able to ubiquitinate mitochondrial proteins including VDAC1, Mfn1, Mfn2 and other proteins[17,18]. Proteins with predominantly K48-linked ubiquitin chains are eliminated by proteosomal degradation. For example, degradation of mitofusins with this modification helped isolating damaged mitochondria from the mitochondrial network by preventing their fusion with healthy mitochondria[19]. Other proteins with K63-linked ubiquitin chains are recognized by p62/SQSTM1, which further recruits autophagosomes through its LC3-binding domain[20].

PINK1 AND PARKIN REGULATE CARDIAC FUNCTION UNDER NORMAL AND STRESSED CONDITIONS

Loss of function mutation in genes encoding Parkin[10] and PINK1[11] was initially identified to be responsible for the pathogenesis of autosomal recessive juvenile forms of Parkinson's disease, and were mostly studied in neurons. However, the vital role of PINK1 and Parkin in hearts is reported at present. According to the recent studies discussed in the present review, PINK1 has a more critical role in maintaining cardiac function under physiological condition whereas Parkin is mainly involved in cardioprotection in response to stresses.

PINK1 is Indispensable for Cardiac Homeostasis

In normal conditions, PINK1 is almost undetectable because of quick processing and degradation. However, present evidence suggests PINK1 is indispensable for cardiac homeostasis.

A study by Billia et al.[21] showed that mice depleted in PINK1 developed cardiac hypertrophy and left ventricular dysfunction as early as 2-months-old, accompanied by higher degrees of cardiomyocytes apoptosis with fibrosis and reduction in capillary density. Furthermore, mitochondria in PINK1-deficient hearts showed swelling morphology, reduced membrane potential and decreased oxidative phosphorylation, resulting in enhanced oxidative stress. Consistently, a decreased protein level of PINK1 was observed in end-stage human heart failure, showing that PINK1 regulates mitochondrial function, ROS production and hypertrophic signaling in the heart.

Loss of PINK1 and Parkin Exacerbates Heart Injury

Kubli et al.[22] recently reported that Parkin-deficient mice were more sensitive to myocardial infarction, as shown by reduced survival and larger infarcts 7 days after the left anterior descending coronary artery ligation surgery, despite normal cardiac function being observed under physiological conditions. Similarly, downregulation of Parkin in cardiac HL-1 cells resulted in a significantly increased extent of cell death after simulated ischemia/reperfusion[23]. In PINK1-deficient cardiomyocytes, antimycin induced higher levels of apoptosis and mitochondrial depolarization[21]. These findings suggest that PINK1 and Parkin play a role in the heart's adaption to stress.

Parkin is Involved in Cardioprotection

Ischemia/reperfusion results in programmed cell death accompanied with impaired autophagy at both the induction and degradation stage[8] in cardiomyocytes, whereas enhancing autophagy through ischemic preconditioning or overexpressing Beclin-1 protect the heart[24] and cardiac HL-1 cells[8] against ischemia/reperfusion injury, respectively. Recently, an emerging role of mitophagy mediated by Parkin in cardioprotection has been discovered.

Huang et al.[23] found ischemic preconditioning was able to induce translocation of Parkin and p62 to depolarized mitochondria in cardiac cells and Langendorff-perfused rat hearts, leading to a depletion of mitochondria through autophagy. As expected, ischemic preconditioning-induced cardioprotection was abolished by Parkin ablation. Furthermore, overexpression

of functional Parkin was found to reduce cell death induced by hypoxia in adult rat cardiomyocytes[22], and increase mitophagy in response to simulated ischemia in cardiac HL-1 cells[23].

PINK1/PARKIN AND DIABETES

There are few studies exploring the link between PINK1/Parkin and diabetes. A study by Scheele *et al.*[3] reported *pink1* transcription was suppressed in the skeletal muscle of type 2 diabetic patients, suggesting a role of PINK1 in glucose metabolism and diabetes despite the mechanisms being unknown. Xu *et al.*[4] recently found that along with a decreased autophagy level, Parkin and PINK1 were also dramatically reduced in type 1 diabetic heart, showing that diabetes might compromise cardiac mitophagy. Intriguingly, in contrast to the general belief that inhibited mitophagy could result from blunted autophagy, that study suggested the attenuated autophagy to be an attempt to activate non-canonical autophagy, which was responsible for mitochondrial elimination in that circumstance.

POTENTIAL INVOLVEMENT OF PINK1 AND PARKIN IN DIABETIC HEART BY MODULATING MITOPHAGY

Autophagy and mitophagy are critical for the maintenance of cardiac function; however, they failed to be induced in Parkin-deficient hearts and isolated cardiomyocytes, respectively, leading to resultant accumulation of damaged mitochondria and

cell death[22], showing that impairment of PINK1/Parkin-dependent mitochondrial elimination through mitophagy could lead to heart injury. Nevertheless, few studies to our knowledge have explored the relationship between PINK1/Parkin-dependent mitophagy and pathogenesis of diabetes-associated cardiac dysfunction.

Mitophagy Imbalance in Diabetic Heart

Autophagy and mitophagy has long been shown to be associated with diabetes. Autophagy undergoes dysregulation in the diabetic heart. It is now suggested that autophagy is suppressed and induced in type 1 and type 2 diabetes, respectively[25]. A study by Mellor *et al.*[26] reported increased cardiac autophagy in a type 2 diabetic mouse model, the same as our unpublished findings that autophagy as well as mitophagy was overacted in the hearts of type 2 diabetic rats. Conversely, Xie *et al.*[27] and Xu *et al.*[4] reported that cardiac autophagy was inhibited in mouse models of type 1 diabetes. Whether autophagy plays a beneficial or detrimental role in the pathogenesis of diabetes is also controversial. Whereas chronically activated autophagy in type 2 diabetes is regarded to confer harm[25], the inhibition of autophagy in type 1 diabetic hearts has recently been proved to be protective[4]. Autophagy is required to eliminate damaged mitochondria, and complex factors are required to accomplish the mitophagy process. It is imaginable that both excessive and blunted mitophagy are harmful. Lacking PINK1/Parkin
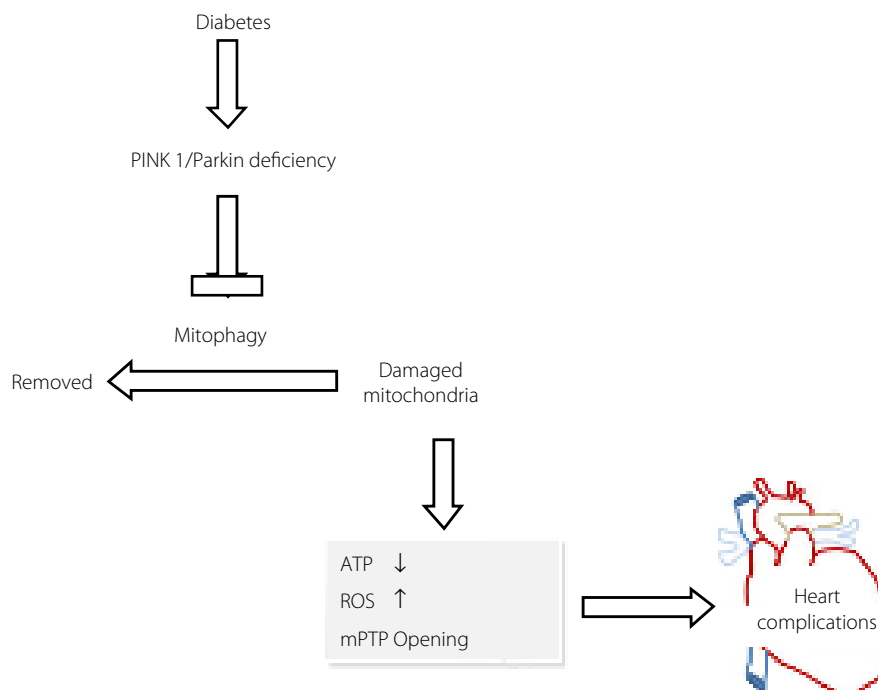

Figure 1 | Proposed paradigm of how phosphatase and tensin homolog-induced putative kinase 1 (PINK1)/Parkin deficiency in diabetic condition can lead to diabetic cardiac dysfunction. ATP, adenosine triphosphate; mPTP, mitochondrial permeability transition pore, ROS, reactive oxygen species.

signaling and mitophagy might be involved in diabetic cardiac complications through some potential mechanisms discussed below.

Potential Mechanisms Contribute to Diabetes-Associated Cardiac Complications Resulted From Mitophagy Deficiency

PINK1/Parkin-mediated mitophagy has a pivotal role in maintaining cardiac function, and impairment of mitophagy might result in the loss of adaption ability and severe cardiac dysfunctions in pathologies including diabetes through some potential mechanisms.

ATP Deprivation

Decline of ATP was found in hearts suffering from stresses[28,29]. Although healthy mitochondria supply the majority of energy, depolarized mitochondria not only produce less ATP, but also hydrolyze ATP in an attempt to restore membrane potential[23]. As a result, selective destruction of damaged mitochondria would maintain ATP content in response to heart injuries. Evidence shows that preventing ATP depletion protected the heart against ischemia/reperfusion injury[28].

ROS Overproduction

ROS are small and highly reactive molecules that can oxidize proteins, lipids and DNA. Low levels of ROS serve as signaling molecules, whereas overproduction of ROS leads to damage, and is involved in diseases including diabetes[30], neurodegenerative diseases[31] and cardiovascular diseases[32].

Mitochondria are the main source of ROS under both physiological and pathological conditions. ROS produced by damaged mitochondria might induce mitophagy to eliminate the dysfunctional mitochondria. Stimulation of autophagy attenuates ROS production in cardiac HL-1 cells exposed to lipopolysaccharide (LPS)[33], whereas deletion of autophagic protein results in defected mitochondrial respiration and increased steady state levels of reactive oxygen species in skeletal muscle[34]. However, enhanced oxidative stress was observed in PINK1[21], but not Parkin[22] knockout heart, suggesting PINK1 and Parkin have a different impact on mitochondrial ROS production under normal conditions.

Mitochondrial Permeability Transition Pore Opening

Opening of the mitochondrial permeability transition pore (mPTP) is a double-edged sword for cell survival decision. On one hand, opening of the mPTP of individual damaged mitochondria is critical to remove dysfunctional mitochondria through autophagy, while failure of this process will lead to an accumulation of damaged mitochondria. On the other hand, constant opening of mPTP leads to release of proapoptotic molecules and triggering of apoptosis. Actually, reducing the open ability of mPTP is able to reverse cardiac apoptosis induced by stresses[35], but can still impair mitophagy in cardiomyocytes under starved conditions[36].

Release of Mitochondrial Deoxyribonucleic Acid

Elimination of damaged mitochondria through autophagy includes removal of mitochondrial DNA, which contains inflammatogenic unmethylated DNA similar to that of bacteria. Inefficient mitophagy at either the initial or late stage results in the release of mitochondrial deoxyribonucleic acid (mtDNA) and inflammatory cytokine expressions by mtDNA in those macrophages and cardiomyocytes.

In macrophages deficient in Beclin-1 and LC3, LPS and ATP synergistically enhanced mtDNA release into the cytoplasm as a consequence of mitochondrial ROS and subsequent mitochondrial membrane transition, leading to activation of caspase-1 and secretion of interleukin-1β and interleukin-18[37]. Oka et al.[9] recently reported another scheme of how mtDNA induces inflammation and heart failure. In this scenario, pressure overload activated, but did not inhibit autophagy in the failing heart. However, as a result of the inactivation of DNase II located in the lysosome, preserved mtDNA lead to Toll-like receptor 9-mediated inflammatory responses in cardiomyocytes.

CONCLUSION

Present studies have shown that PINK1 and Parkin interfere in controlling cardiac function, which might involve mitophagy. Based on the additional fact that PINK1 and Parkin are reduced in both type 1 and type 2 diabetes, we propose that PINK1/Parkin could play a role in diabetic heart dysfunctions, though there is no evidence up to now. To elucidate this, the first question that needs to be answered is whether and how PINK1/Parkin can be influenced by diabetes in the heart. Second, uncovering the mechanisms that contribute to PINK1/Parkin-mediated diabetic cardiac complications is of importance. In the present review, we mainly focus on PINK1/Parkin-dependent mitophagy (Figure 1).

Adequate mitophagy guards heart function and adaption to injuries by degrading aged and damaged mitochondria. Cardiomyocytes deficient in PINK1 and Parkin, two critical mitophagy regulators, show defects under normal and stressed conditions, accompanied by inhibition of autophagy/mitophagy and accumulation of dysfunctional mitochondria[21,30]. As a result, the heart will suffer from less production of ATP and overproduction of ROS. Opening of mPTP might also induce damage in the heart through releasing proapoptotic molecules and inflammatogenic mtDNA. All these are potential mechanisms responsible for diabetic heart dysfunction induced by impaired mitophagy.

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