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Sphingosylphosphorylcholine protects cardiomyocytes against ischemic apoptosis via lipid raft/PTEN/Akt1/mTOR mediated autophagy

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ABSTRACT

Autophagy, evoked by diverse stresses including myocardial ischemia/reperfusion (I/R), profoundly affects the development of heart failure. However, the specific molecular basis of autophagy remains to be elucidated. Here we report that sphingosylphosphorylcholine (SPC), a bioactive sphingolipid, significantly suppressed apoptosis and induced autophagy in cardiomyocytes. Blocking this SPC evoked autophagy by 3-methyladenine (3MA)-sensitized cardiomyocytes to serum deprivation-induced apoptosis. Subsequent studies revealed that SPC downregulated the phosphorylation of p70S6K and 4EBP1 (two substrates of mTOR) but enhanced that of JNK when inducing autophagy. We identified SPC as a switch for the activity of Akt1, a supposed upstream modulator of both mTOR and JNK. Furthermore, β-cyclodextrin, which destroys membrane cholesterol, abolished the SPC-reduced phosphorylation of both Akt and PTEN, thus inhibiting SPC-induced autophagy. In conclusion, SPC is a novel molecule protecting cardiomyocytes against apoptosis by promoting autophagy. The lipid raft/PTEN/Akt1/mTOR signal pathway is the underlying mechanism and might provide novel targets for cardiac failure therapy.

1. Introduction

Sphingosylphosphorylcholine (SPC), a bioactive sphingolipid, is naturally occurring in blood plasma or is produced by the intermediary metabolism of sphingomyelin [1]. Since SPC was identified as a significant constituent of lipoproteins [2], its function in the cardiovascular system has attracted extensive attention. SPC affects proliferation, apoptosis and differentiation of endothelial cells [3–5], cytoskeleton rearrangement and contraction of vascular smooth muscle cells [6,7], and hypertrophic growth and apoptosis of cardiomyocytes [8,9]. Hence, SPC is a promising regulatory molecule in the cardiovascular system. We previously reported that SPC could induce anti-apoptotic autophagy in human umbilical vein endothelial cells and non-small lung cancer cells [10,11]. We wondered about the effect of SPC on apoptosis and autophagy in cardiomyocytes.

Cardiomyocytes are terminally differentiated, and stress-induced loss of those cells will be detrimental to the heart. Autophagy plays a fundamental role in the maintenance of cardiomyocyte homeostasis. Under stressed conditions, autophagy is further evoked to perform specific functions in the impaired heart. Autophagy, activated during myocardial ischemia/reperfusion (I/R), protects the heart during ischemia but may become harmful during reperfusion [12]. The elucidation of a novel regulator of cardiomyocyte autophagy and the corresponding mechanisms holds promise for identifying new therapeutic avenues for myocardial injury.

Mammalian target of rapamycin (mTOR), a serine/threonine kinase, is a key modulator of autophagy in mammalian cells. By associating with different proteins, mTOR molecules are assembled into two structurally distinct multiprotein complexes, mTOR complexes 1 and 2 (mTORC1 and mTORC2). Activated mTORC1 promotes the protein synthesis by phosphorylating its substrates p70S6K and 4EBP1 but negatively regulates autophagy. Pharmacological restraint of mTORC1 maintains and improves cardiac function in diverse cardiac-related diseases including myocardial infarction [13]. Whether SPC can affect mTOR-dependent autophagy in cardiomyocytes still needs to be investigated.

MTORC1 activity relies on the regulation of Akt [14], which is widely involved in cell survival and autophagy modulation. Akt is essential during postnatal cardiac development [15]. The short-term activation of Akt has beneficial effects on the heart via inhibiting apoptotic cell

Abbreviations: SPC, sphingosylphosphorylcholine; I/R, ischemia/reperfusion; MTOR, mammalian target of rapamycin; MTORC1, mTOR complex 1; MTORC2, mTOR complex 2; MI, myocardial infarction; PI3K, phosphatidylinositol 3-kinase; TSC2, tuberous sclerosis complex 2; HUVECs, human umbilical vein endothelial cells; SCaMPER, sphingolipid calcium release-mediating protein of the endoplasmic reticulum.

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death and improving contractile function, whereas long-term Akt activation induced pathological hypertrophy [16]. Three isoforms of Akt exist in mammals: Akt1, Akt2 and Akt3. Most previous studies elaborated the function of Akt regardless of subtype specificity [17].

In the present research, we investigated whether SPC could protect cardiomyocytes against apoptosis by inducing mTOR-dependent autophagy and the underlying mechanism. We also further determined the isoform-specific role of Akt in cardiomyocyte autophagy.

2. Materials and methods

2.1. Reagents

SPC was from Sigma (St. Louis, MO, USA) and dissolved in ethanol at 0.01 M as a stock solution. Dulbecco's modified Eagle's medium (DMEM) was from Gibco (Grand Island, NY, USA). Fetal bovine serum (FBS) was from Hyclone Lab (Logan, UT). Dimethyl sulfoxide (DMSO), Bafilomycin A1, protease inhibitor cocktail, 3-methyladenine (3-MA) and JNK inhibitor SP600125 were from Sigma Aldrich (St. Louis, MO, USA). β-cyclodextrin was from Sangon Biotech (Shanghai, China). An Annexin V-FITC Apoptosis Detection Kit was purchased from BioLegend (California, USA). GFP-LC3 constructs were from addgene (USA). Q3

2.2. Cell culture and treatment

Neonatal rat cardiomyocytes (NRCMs) were isolated from the heart of SD rats born within 3 days. The hearts were minced and digested in a mixture of trypsin and Type II collagen enzyme. Primary neonate rat cardiomyocytes were cultured in DMEM containing 10% FBS, penicillin and streptomycin. To mimic the ischemic damage, cells were cultured in DMEM deprived of glucose and placed in a hypoxic incubator (95% N_2, 5% CO_2, 37 °C) for 4 h in the presence of SPC or equal concentration of ethanol.

Rat myocardium-derived H9c2 cardiomyocytes were cultured in DMEM with 10% FBS at 37 °C in humidified air and 5% CO_2. During the experiment, H9c2 cells were incubated in DMEM medium deprived of

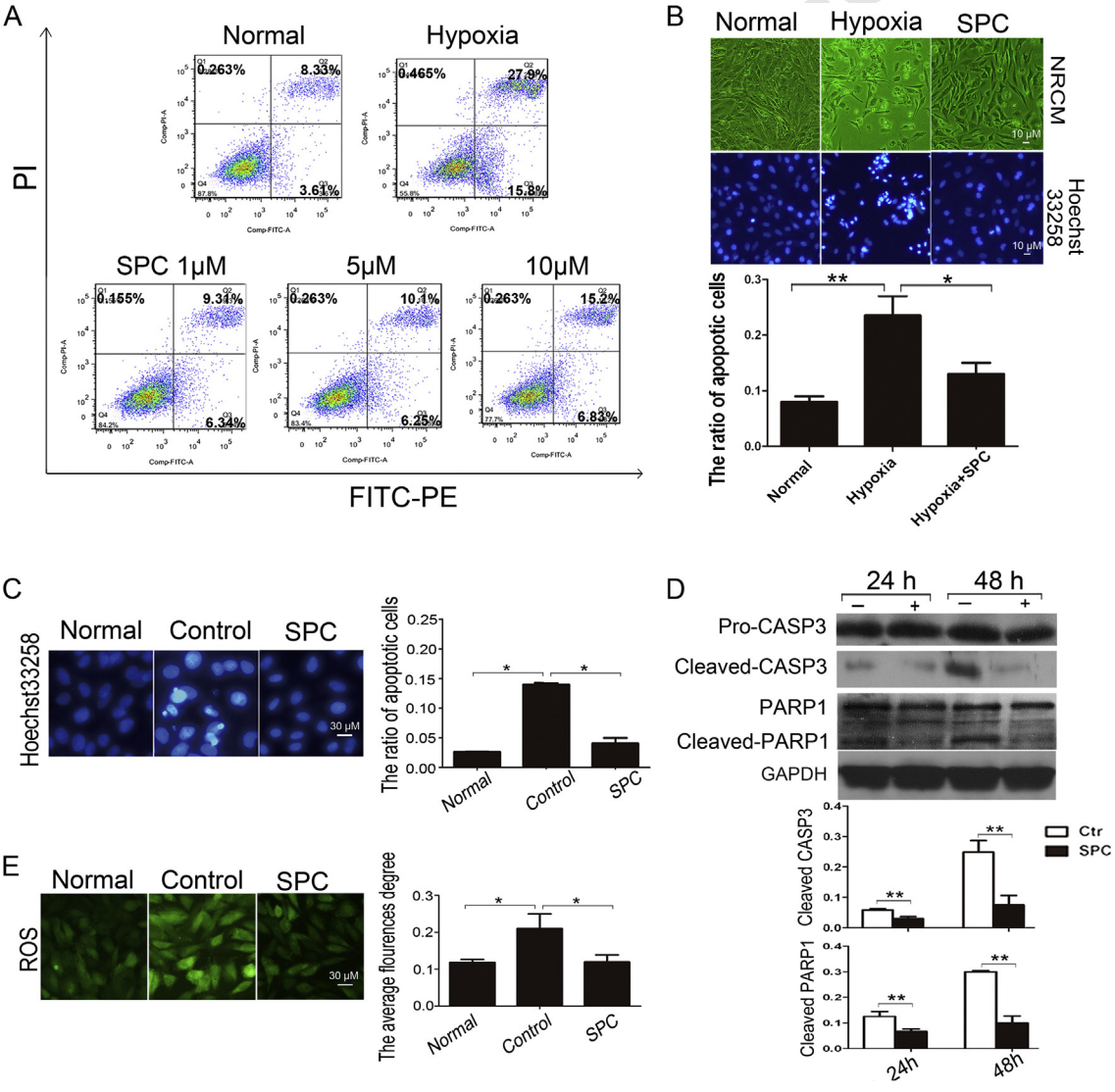

Fig. 1. Sphingosylphosphorylcholine (SPC) protected cardiomyocytes against apoptosis. Neonatal rat cardiomyocytes (NRCMs) were exposed to hypoxia with or without SPC for 4 h. (A) ANXA5-PE/PI staining and flow cytometry analysis of the apoptotic ratio of NRCMs. (B) Hoechst 33258 staining of DNA fragmentation and condensation in NRCMs. H9c2 cells were cultured in serum-free medium with or without SPC for indicated times. Cells treated with ethanol were controls. Scale bar = 10 μM. (C) H9c2 cells were treated with 10 μM SPC for 24 h. Hoechst 33258 staining of DNA fragmentation and condensation of H9c2 cells. Scale bar = 30 μM. (D) Western blot analysis of protein levels of apoptosis-related proteins caspase3 and poly (ADP-ribose) polymerase 1 (PARP1). (E) H9c2 cells were treated with 10 μM SPC for 24 h. The intracellular ROS level was detected by a DCFD probe. Scale bar = 30 μM. $^{**}P < 0.01$, $^{*}P < 0.05$; n = 3.

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