Supplementary Materials and methods

MTT assay

Cells were seeded in 96-well plates (1×10^5 cells/mL). The cell growth determination kit (MTT based) (Sigma-Aldrich, Missouri, USA) was used according to the manufacturer's instructions. THERMO FISHER Multiskan FC (Thermo Fischer Scientific, Waltham, MA, USA) was used to assess the absorbance at 570 nm.

RNA isolation and qRT-PCR analysis

Total RNA was extracted using TRizol reagent (Invitrogen, USA). The reverse transcription was performed by using PrimeScript RT reagent Kit with gDNA Eraser (Takara, Japan). QRT-PCR was performed by using SYBR Green qPCR Master Mix (Takara). Stem-loop qRT-PCR for mature miR-421 was performed with TaqMan miRNA probes (Applied Biosystems, USA). The housekeeping gene GADPH and U6 were used as an internal standard. Relative amount of transcripts were quantitated using the Ct formula. The primers used are listed in Table 1.

Western blotting assay

Total protein was extracted using RIPA lysate, and protein concentration was determined by BCA protein concentration assay kit (Bio-Rad, USA). Each well was loaded with 50 μg of sample and subjected to SDS-PAGE electrophoresis. After PVDF membrane (Millipore; USA) transfer, 5% skim milk powder was added for 2 h, primary antibody was added and incubated at 4 °C overnight. Then incubated the membrane with the secondary antibody (ZSGB-BIO, China). Visualization was performed by using ECL assay (Millipore). The antibodies used in this study included anti-GAPDH (Abcam, USA), anti-Sirt3 (Cell Signaling Technology, USA),

anti-cleaved caspase-9 (Abcam), anti-cleaved caspase-3 (Abcam), anti-c-Jun (Cell Signaling Technology), anti-JNK (Cell Signaling Technology), anti-phosphorylated-c-Jun (p-c-Jun; Cell Signaling Technology), anti-phosphorylated-JNK (p-JNK; Cell Signaling Technology), anti-AP-1 (Cell Signaling Technology), anti-Bax (Abcam), anti-Bcl-2 (Abcam).

Detection of lactate dehydrogenase (LDH), superoxide dismutase (SOD) and malondialdehyde (MDA) expression levels

According to the instructions of the LDH assay kit (Invitrogen, USA), the cell culture supernatants of each group were collected and the corresponding reagents were added. The microplate reader measures the absorbance at a wavelength of 450 nm. LDH release was expressed in units per liter (U/L).

The cells were digested with 0.25% trypsin and centrifuged, and operated according to the SOD and MDA assay kit (Invitrogen) instructions. The microplate reader measures the absorbance at 450 and 530 nm, respectively. The total amounts of SOD and MDA were expressed in U/mg and mmol/mg.