Scientific research process

Until now, few studies have mentioned about Remote ischemic perconditioning (RIC) in liver ischemia-reperfusion injury (IRI) and most of them only confirmed the protective effect of RIC, but few of them showed detailed mechanism. These circumstances have led to its mechanism remains largely unknown. In previous studies, we have optimized the application of RIC and demonstrate its protections against IRI. In this study, we further investigated the underlying mechanisms of it.

Ca^{2+} plays an important role in apoptosis. In our previous RIC experiment, compared with the OLT group, RIC group showed decreased apoptosis. In the present study, we hypothesized and verified whether RIC model via Mfn2-MICUs axis has protective effect for LT. As it is hard to perform gene knockout operations using primary cells, genetically engineered mice are expensive and experiment is time-consuming, we designed the use of AML12 hypoxia cell lines to simulate RIC model and to revalidate it to prove our hypothesis.

The Sprague–Dawley rats were divided into three groups (n=6 each): sham, orthotopic liver transplantation (OLT) and remote ischemic perconditioning (RIC). After operation, blood samples were collected to test alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The liver lobes were harvested for histopathological examination, Western blotting (WB) and quantitative real-time polymerase chain reaction (qRT-PCR). AML12 cell lines were then subjected to normal culture (NC), anoxic incubator tank culture (Hypoxia) and anoxic incubator tank culture with Mfn2 knockdown (Hypoxia+Si), and data of qRT-PCR, WB, mitochondrial membrane potential ($\Delta\Psi m$), apoptosis, endoplasmic reticulum (ER) Ca^{2+} concentrations and mitochondrial Ca^{2+} concentrations were collected. The results were expressed as mean ± SEM. The one-way analysis of variance (ANOVA) was used for comparisons among three groups and the *t* test was used for comparison between the two groups.

The result verified our hypothesis. We used an RIC model and confirmed that IRI was prevented by altered organelles Ca^{2+} status via Mfn2-MICUs axis, and revalidated the effect in AML12 hypoxia cell models.

To our knowledge, this research is the first to prove the protective mechanism of MFN2-MICUs axis by affecting the metabolism of intracellular calcium in RIC model of liver transplantation and revalidate it in AML12 hypoxia cell lines, showed the relationship between the impacts of Mfn2-MICUs axis, the protective effect of RIC/cell hypoxia injury and the apoptosis which affected by the intracellular calcium homeostasis, thus laying a basic for future studies.