

Title: HMGB1/Caspase-8/RIP3 Axis Mediates Mitochondrial Apoptosis in Septic Cardiac Microvascular Endothelial Injury

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Background: Sepsis-induced cardiac microcirculation disturbance significantly causes cardiac dysfunction and increased mortality. Cardiac microvascular endothelial cells (CMECs) closely interact with cardiomyocytes, and endothelial injury is a critical link in sepsis-induced cardiac injury. HMGB1 and caspase-3 are elevated in sepsis, potentially inducing mitochondrial dysfunction and subsequent apoptosis signaling, but the specific mechanism remains unclear in CMECs.

Materials and Methods: A mouse sepsis model was established via intraperitoneal LPS injection. Endothelial-specific HMGB1 knockout mice were used for in vivo assessment. CMECs were isolated and cultured for in vitro experiments. HMGB1, RIP3, and caspase-8 were detected by Western blot and immunofluorescence. Microvessel density, caspase-3 activity, and cardiac function were evaluated by CD31 staining, activity assay, and echocardiography, respectively. Mitochondrial

ROS and fragmentation were assessed via MitoSOX and live-cell labeling. Apoptosis was evaluated by TUNEL and cleaved caspase-3/PARP detection.

Results: Sepsis significantly increased HMGB1 and RIP3 in CMECs, decreased microvessel density, and worsened cardiac function. At the gene level, HMGB1 modulated RIP3 splicing, reducing the splice variant RIP3 γ and relatively increasing full-length RIP3. At the protein level, HMGB1 down-regulated caspase-8, attenuating RIP3 post-translational cleavage and promoting its mitochondrial accumulation. This mitochondrial anchoring of RIP3 activated mitochondrial apoptosis, driving excessive fission, ROS production, and caspase-3-dependent apoptosis. Endothelial HMGB1 knockout alleviated these pathological changes.

Conclusions: This study reveals a novel dual regulatory mechanism in septic cardiac microcirculatory dysfunction. At the gene level, HMGB1 modulates RIP3 splicing to reduce RIP3 γ . At the protein level, HMGB1 down-regulates caspase-8 to attenuate RIP3 cleavage. This dual regulation promotes RIP3 mitochondrial anchoring, driving excessive fission and ROS production, ultimately activating caspase-3-dependent apoptosis in CMECs and leading to cardiac dysfunction.