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Category: Staff/Tech/Other
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Title: Novel Role of Mitochondria Associated Endoplamic Reticulum Membrane (MAM) Proteins BiP/GRP78 and Mortalin/GRP75 in Mediating Mitochondrial Dysfunction in Endothelial cells
Abstract: Communication between endoplasmic reticulum (ER) and mitochondrion, two multifunctional organelles, is central to many cellular processes such as Ca2+ homeostasis, energy metabolism, lipid metabolism and cell survival. This is achieved through a specialized region of close contact (~10-25 nm) between ER and mitochondria termed as MAM. MAM harbor many chaperones and several key Ca2+ channels that play a pivotal role in forming the ER-Mitochondrion (ER-MITO) contact sites which are critical for signaling pathways essential to maintain cellular homeostasis. Disruption of MAM integrity is implicated in the progression of diseases associated with high inflammation, mitochondrial dysfunction and perturbation in intracellular Ca2+ homeostasis such as metabolic disorders and neurodegenerative diseases. Earlier, we have established the role of two MAM proteins BiP/GRP78 (resides on the ER side of MAM) and mortalin/GRP75 (resides on the mitochondrial side of MAM) in mediating thrombin-induced endothelial cell (EC) permeability associated with acute lung injury (ALI). In the present study we monitored the role BiP/GRP78 and mortalin/GRP75 in regulating ER-MITO contact sites, and mitochondrial Ca2+, ROS, and ATP production in EC. We first confirmed the localization of BiP/GRP78 and mortalin/GRP75 in the MAMs using human pulmonary artery endothelial cells (HPAEC). We next examined the role of BiP/GRP78 and mortalin/GRP75 in regulating ER-MITO contact sites by proximity ligation assay (PLA) using VDAC1 (mitochondria) and IP3R3 (ER) antibodies as probes. Proinflammatory mediator thrombin increased the number of ER-MITO contact sites and inhibition of BiP/GRP78 using HA15 or mortalin/GRP75 using MKT-077, each prevented this response. Intriguingly, both basal and thrombin-induced ER-MITO contact sites were also inhibited by the cell membrane permeant BAPTA-AM which chelates intracellular Ca2+. These data suggest that thrombin-induced increase in ER-MITO contact sites is mediated by BiP/GRP78 and mortalin/GRP75 in part via their ability to regulate Ca2+ signaling. Consistent with this possibility, we show that siRNA-mediated depletion of BiP/GRP78 and Mortalin/GRP75, significantly inhibited thrombin-induced mitochondrial Ca2+ uptake. Furthermore, inactivation of BiP/GRP78 and mortalin/GRP75 decreased mitochondrial ROS generation and ATP production caused by thrombin, implicating a role for BiP/GRP78 and mortalin/GRP75 in mitochondrial dysfunction. Together, our results implicate a novel role of BiP/GRP78 and mortalin/GRP75 in mediating inflammatory signaling via MAM.