Proteomic Analysis and Biochemical Correlates of Mitochondrial Dysfunction following Low-Intensity Primary Blast Exposure

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Overview

Service members during military actions or combat training are frequently exposed to primary blasts by weaponry. Most studies have investigated moderate or severe brain injuries from blasts generating overpressures over 100-kPa, while understanding the pathophysiology of low-intensity blast (LIB)-induced mild traumatic brain injury (mTBI) leading to neurological deficits remains elusive. Our recent studies, using an **open-field LIB-induced mTBI mouse model with an peak overpressure at 46.6-kPa**, demonstrated behavioral impairments and brain nanoscale damages, notably mitochondrial and axonal ultrastructural changes. In this study, we used **tandem mass tagged (TMT) quantitative proteomics and bioinformatics** analysis to seek insights into the molecular mechanisms underlying ultrastructural pathology. Changes in global- and phospho-proteomes were determined at 3 and 24 hours, 7 and 30 days post injury (DPI), and to investigate the biochemical and molecular correlates of mitochondrial dysfunction. Results showed striking dynamic changes in a total of 2216 global and 459 phosphorylated proteins at vary time points after blast. Disruption of key canonical pathways included evidence of mitochondrial dysfunction, oxidative stress, axonal/cytoskeletal/synaptic dysregulation, and neurodegeneration. Bioinformatic analysis identified blast induced trends in networks related to cellular growth/development/movement/assembly and cell-to-cell signaling interactions. With observations of proteomic changes, we found LIB-induced oxidative stress associated with mitochondrial dysfunction mainly at 7 and 30 DPI. These dysfunctions included **impaired fission-fusion dynamics, diminished mitophagy, decreased oxidative phosphorylation, and compensated respiration-relevant enzyme activities**. Insights on the early pathogenesis of primary LIB-induced brain damage provide a template for further characterization of its chronic effects, identification of potential biomarkers and targets for intervention.

Results

Open-field LIB settings to induce mTBI in mice, blast pressure traces, and shockwave impulse

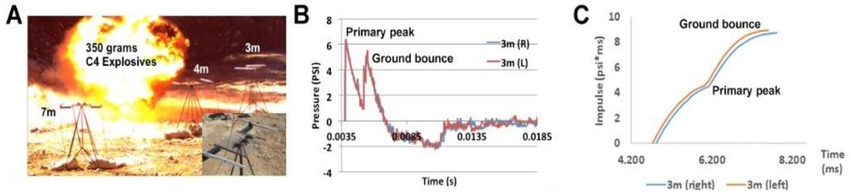

Figure 1. (A) Representation of the blast onset captured by a Blaster's Ranger II 2500-fps high-speed video camera. (B-C) Peak pressure vs. time and Time vs. impulse curves at 3-m distance indicate the overall impact of the dynamic impulse from both the primary peak and ground bounce of blast shockwaves.

Quantitative proteomics analysis of primary LIB effects

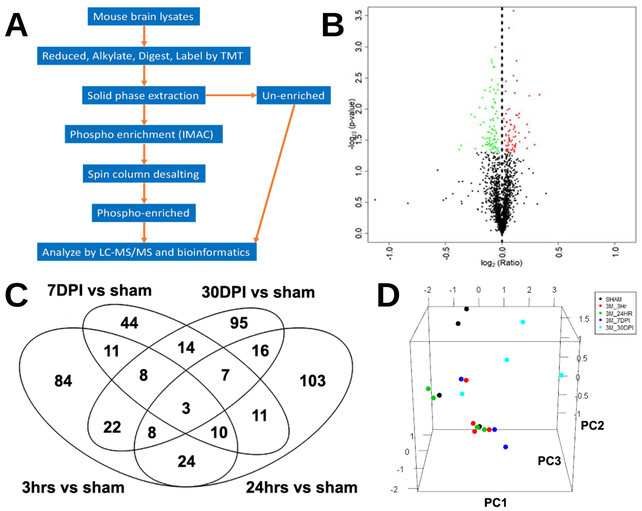

Figure 2. (A) Workflow for quantitative profiling. (B) Volcano plot showing differentially expressed proteins at 3hrs after LIB. (C) Venn diagram showing the overlapping differentially expressed proteins. (D) Principle component analysis visualize the convergence and/or divergence of proteome changes among groups.

Cellular components and signaling pathways enriched after LIB injury

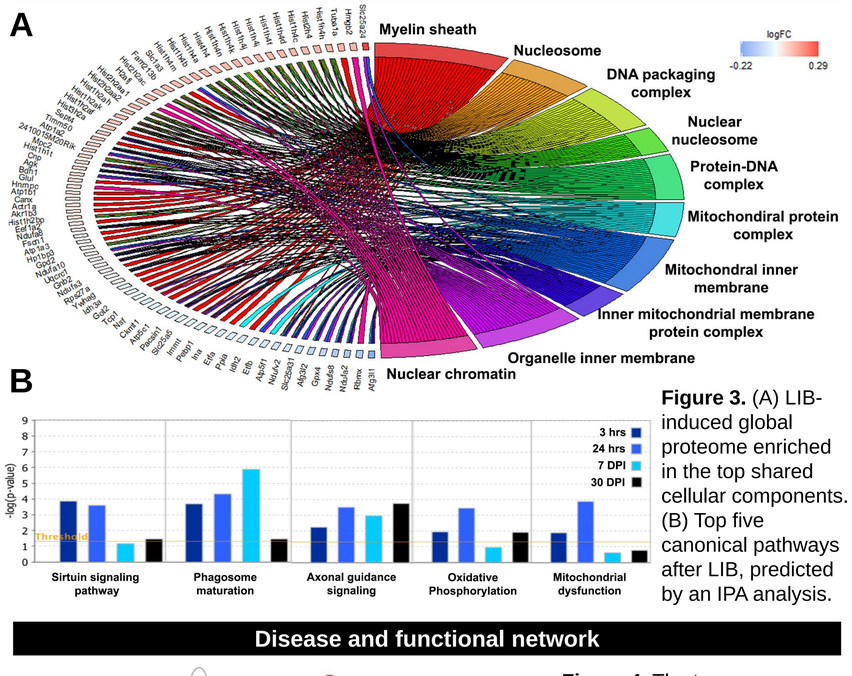

Figure 3. (A) LIB-induced global proteome enriched in the top shared cellular components. (B) Top five canonical pathways after LIB, predicted by an IPA analysis.

Disease and functional network

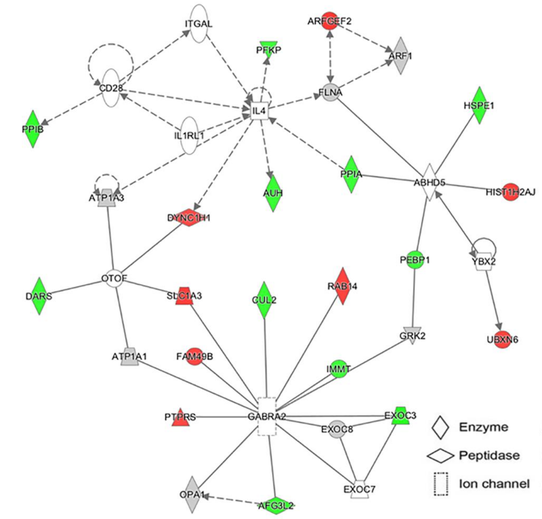

Figure 4. The top disease/function network in global-proteome is associated with cellular development, cellular growth and proliferation, cellular movement, and cellular assembly and organization corresponding to the LIB.

LIB-induced mitochondrial dysfunction associated with oxidative stress and fission-fusion dynamics

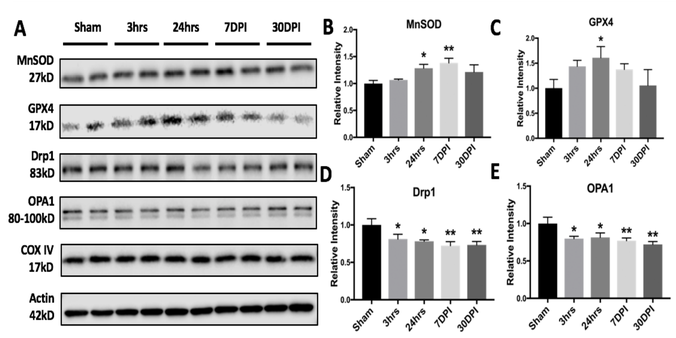

Figure 5. Western blot and analysis of mitochondrial markers, including MnSOD, GPX4, Drp1, and OPA1, associated with oxidative stress and fission-fusion dynamics after LIB.

LIB-induced oxidative phosphorylation and bioenergetic impairment

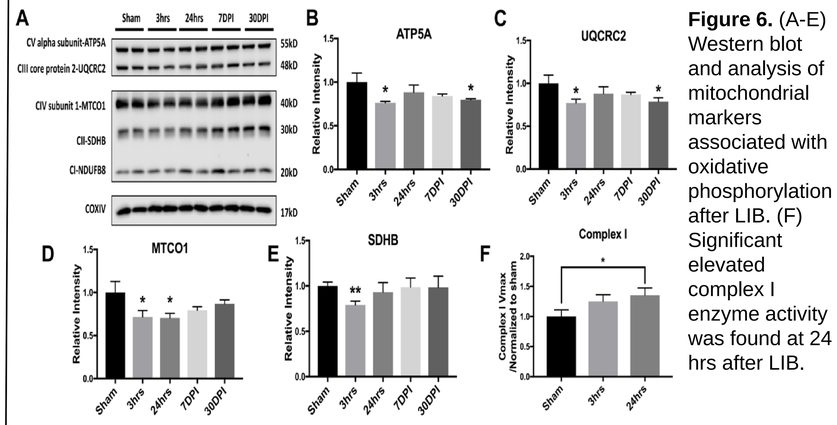

Figure 6. (A-E) Western blot and analysis of mitochondrial markers associated with oxidative phosphorylation after LIB. (F) Significant elevated complex I enzyme activity was found at 24 hrs after LIB.

LIB-induced mitochondrial ultrastructural abnormalities

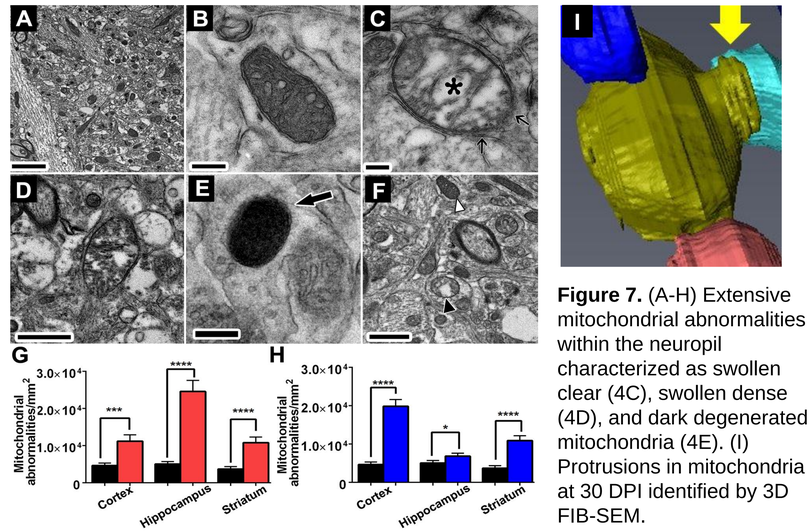

Figure 7. (A-H) Extensive mitochondrial abnormalities within the neuropil characterized as swollen clear (4C), swollen dense (4D), and dark degenerated mitochondria (4E). (I) Protrusions in mitochondria at 30 DPI identified by 3D FIB-SEM.

Conclusions

Our observations demonstrate that primary LIB injury comprises an 'invisible' injury in the presence of nanoscale myelinated axonal injury and mitochondrial damages associated with neurobehavioral impairments. Further, we found LIB induced oxidative stress associated with mitochondrial dysfunction mainly at 7 and 30 DPI. Our study provides key insights into the pathogenesis and potential treatment of primary LIB injury.

References

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