Proteome profiling of C2C12 myotubes with alternated insulin sensitivity upon palmitic acid treatment

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Obesity has a tight association with type 2 diabetes mellitus (T2DM) and elevated plasma free fatty acid level induced insulin resistance is believed as the link between obesity and T2DM. However, the detailed mechanism of the changes in plasma free fatty acid level result in insulin resistance remains to be elucidated. In this study, insulin desensitization was induced in C2C12 myotubes via palmitic acid treatment. To focus on the changes of nuclear proteome, nuclei of C2C12 myotubes were isolated for two-dimensional gel electrophoresis based proteomic study. Result demonstrated that four nuclear proteins showed changes in expression after palmitic acid treatment; nuclear factor NF-kappa-B (NF-κB) p65 subunit and 60S acidic ribosomal protein P0 were upregulated, while peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC-1α) and cleavage and polyadenylation specificity factor subunit 5 (CFIm25) were downregulated. Whereas, inhibiting NF-κB p65 subunit nuclear translocation can prevent the palmitic acid induced deleterious effect on insulin sensitivity, implied that NF-κB p65 subunit play a key role in palmitic acid induced insulin desensitization.

Methods: A murine skeletal muscle cell line, C2C12 myotubes were established and exposed to first, palmitic acid in order to induce insulin desensitization; and followed by treatment with oleic acid to act as control. To focus on the changes of nuclear proteome in comparing with that of the cytosolic proteomic status, nuclear fractions were enriched by centrifugation for two-dimensional gel electrophoresis (2-DE) based proteomic study.

Results & Discussion: The 2-DE result was confirmed by western blotting analysis Five differentially expressed proteins were found. After 24 h fatty acid treatment, nuclear fractions were enriched and applied to 2-DE. Five proteins demonstrated changes in expression after palmitic acid treatment. Among these five proteins, nuclear factor-kappa-B (NF-κB) p65 subunit and 60S acidic ribosomal protein P0 were upregulated, after exposed to plamitic acid; while peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC-1α), cleavage and polyadenylation specificity factor subunit 5 (CFIm25) and prohibitin were downregulated. Inhibiting NF-κB activation could rescue C2C12 myotubes from palmitic acid induced insulin desensitization. Inhibiting NF-κB activation by parthenolide reversed the deleterious effects of palmitic acid on Akt activation and insulin stimulated glucose uptake .These results indicated that NF-κB p65 subunit was involved in palmitic acid induced insulin desensitization.

Biography

Ngai Sai Ming is currently Director of The Chinese Medicinal Fungal Proteomics Laboratory and Investigator of State Key Laboratory for Agrobiotechnology and associate professsor in The School of Life Sciences, in The Chinese University of Hong Kong, Hong Kong SAR, China. His research interest is bioinformatics, proteomics and metabolomics, protein/peptide structural and functional studies, and Modern Chinese Medicine. He has over 20 years experience in Protein/Peptide biochemistry, proteomics and computational techniques and is the author of over 70 scientific publications, 4 book chaperters and numerous conference papers.

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