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Gene Expression Profiling on Skeletal Muscle after Exercise Training

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Obesity has been associated with insulin resistance in skeletal muscle; accordingly, exercise dramatically improves insulin action. We sought to identify molecular remodeling of muscle commensurate with exercise training that could explain improvements in insulin action. Three women participated in 9 months of aerobic exercise training at 65-80% of VO2max. The range of age was from 40 to 50 years old and BMI (Body Mass Index) was from 25 to 35. They were sedentary and had hyperinsulinemia with lipid abnormalities. Muscle biopsy (vastus lateralis) was taken before and after exercise training (24 hr after the last bout of exercise). We assessed mRNA expression profiles using Affymetrix U133 Plus 2.0 array, with a stringent statistical analysis (statistical concordance with three probe set algorithms). We also validated the expression of selected genes using RT-PCR, and used Ingenuity Pathways Analysis (http://www. ingenuity.com) to determine functional relationships among the identified genes. Exercise training altered expression of 71 genes in skeletal muscle. We found that these genes were involved in 4 significant molecular networks, 11 biological functions, and 10 canonical pathways. Of these, genes related with energy (ATP) production, carbohydrate metabolism, and molecular transport were all increased. In particular, mitochondrial complex I-V genes increased their expression. Therefore, these results suggest that exercise enhances muscle metabolism through increasing oxidative phosphorylation, thereby preventing the development of insulin resistance.

Key words: Gene expression profiling, exercise, muscle

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Multi-Functional Role of Rottlerin, PKC-Delta Inhibitor, in Immune Cells and Cancer Cells

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Rottlerin, a compound reported to be a PKC δ-selective inhibitor, has been shown to induce growth arrest or apoptosis of human cancer cell lines. In our study, rottlerin dose-dependently induced apoptotic cell death in colon carcinoma cells. Treatment of HT29 human colon carcinoma cells with rottlerin was found to induce a number of signature ER stress markers; phosphorylation of eukaryotic initiation factor-2α (eIF-2α), ER stress-specific XBP1 splicing, and up-regulation of glucose-regulated protein (GRP)-78 and CCAAT/enhancer-binding protein-homologous protein (CHOP). However, suppression of PKC δ expression by siRNA or overexpression of WT-PKC δ and DN-PKC δ did not abrogate the rottlerin-mediated induction of CHOP. We found that treatment with rottlerin significantly induces DR5 expression both at its mRNA and protein levels but not death receptor 4. Down-regulation of DR5 expression with siRNA efficiently attenuated rottlerin-induced apoptosis, showing that the critical role of DR5 in this cell death. Rottlerin-induced DR5 upregulation was accompanied by CCAAT/enhancer-binding protein-homologous protein (CHOP) protein expression. Not only suppression of PKC δ expression by siRNA but also overexpression of WT-PKC δ or DN-PKC δ did not affect the rottlerin-mediated induction of DR5 in our study. These results suggest that rottlerin induces up-regulation of DR5 via PKC δ-independent pathway. Furthermore, subtoxic dose of rottlerin sensitizes human cancer cells to TRAIL-mediated apoptosis. Thus, DR5-mediated apoptosis, which is induced by rottlerin alone or by the combined treatment with rottlerin and TRAIL, may offer a new therapeutic strategy against cancer. We investigated that rottlerin induced the expression of heme oxygenase-1 (HO-1), which is known to modulate various cellular functions, including cytokine production, cell proliferation, and apoptosis, in stress-related condition. Rottlerin increased ROS generation and ROS scavenger, GSH or NAC inhibited HO-1 expression. We investigated that p38 MAPK inhibitor, SB203580 inhibited rottlerin-induced HO-1 expression. Rottlerin increased translocation of Nrf-2 into nucleus and ARE-luciferase activity, which are inhibited by ROS scavengers or p38 inhibitor. And suppression of PKC δ expression by siRNA or overexpression of WT-PKC δ and DN-PKC δ did not abrogate the rottlerin-mediated induction of HO-1. These data suggest that rottlerin induces up-regulation of HO-1 through transactivation of Nrf2 in ROS- or p38 MAPK-dependent mechanism.

Key words: Rottlerin, DR5, CHOP, ER stress, HO-1