Suppression of NDRG2, a novel PGC-1α and PGC-1β target gene, contributes to the metabolic profile and protein synthesis rates of skeletal muscle cells

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N-myc downstream-regulated gene 2 (NDRG2) controls cellular proliferation in many cell types including skeletal muscle cells where its suppression results in reduced myoblast proliferation (Foletta *et al*, 2009). Its role in terminally differentiated muscle cells is unknown at present. Recently, however, we identified NDRG2 as a novel target of the transcriptional co-activators, PGC-1α and PGC-1β, in mouse C2C12 myotubes where NDRG2 expression was found induced by both co-activators. The PGC-1α and -1β proteins are key promoters of oxidative metabolism and of myosin genes associated with slow twitch fibre phenotypes. In addition, they help maintain muscle mass by positively regulating protein turnover. Therefore, we characterized the effect of suppressed NDRG2 levels in C2C12 myotubes in relation to these endpoints. We profiled global gene and protein expression changes using DNA microarray and iTRAQ proteomic platforms (AGRF, Melbourne and Proteomics International, Perth, respectively). Gene-set enrichment analytical approaches of both the gene and protein datasets revealed significant associations with biological processes and molecular functions including muscle growth and contraction, ribosome activity, MAPK signaling, oxidative phosphorylation and other mitochondrial-linked metabolic functions. Moreover, intersect analyses of microarray datasets derived from C2C12 myotubes expressing suppressed levels of NDRG2 or increased levels of either PGC-1α or PGC-1β, identified at least 70% of co-regulated genes to be induced similarly following each gene treatment. Further investigation into fibre-type characterization confirmed that reduced NDRG2 expression altered the expression levels of multiple myosin heavy and light chain molecules with a clear suppression evident of the embryonic myosin, Myh3; the latter an important marker of muscle development and regeneration. Finally, protein turnover studies revealed an increase in protein synthesis rates during basal and insulin-stimulated conditions with concurrent increases in the phosphorylation status of p44/p42 MAPK and 4E-BP1 proteins indicating their potential contribution to the increase in protein synthesis. These findings help confirm associations between the functions of NDRG2 and the PGC-1α and -1β proteins. However, in contrast to PGC-1α and -1β, these findings suggest that NDRG2 may behave as a negative modulator of these functions. This study highlights novel biological roles for NDRG2 in differentiated muscle cells linked to PGC-1α- and PGC-1β-related activities.

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