

COMPARATIVE PROTEOMIC ANALYSIS OF NORMAL AND NQO1-NULL MOUSE EMBRYO FIBROBLASTS

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NAD(P)H-quinone oxidoreductase 1 (NQO1) is an ubiquitous cytosolic flavoenzyme that catalyzes two-electron reduction of various quinones, with NADH or NADPH as electron donors. The reduction of quinones prevents their participation in redox-cycling and the subsequent generation of reactive oxygen species (ROS). Reduced apoptosis and increased susceptibility to induced tumors and myeloid hyperplasia have been described in NQO1-null (NQO1^{-/-}) mice. Thus, NQO1 is involved in the protection against oxidative stress and carcinogenesis. The purpose of the present study was to analyze changes in the levels of proteins of cell lysates obtained from control mouse embryo fibroblasts (MEFs) compared with NQO1^{-/-} MEFs. This approach could give us new insights of the functions of NQO1 in cell growth control. In this work, we have used 2D-PAGE coupled to MALDI-TOF mass spectrometry for the identification of proteins which are differentially expressed between control and NQO1^{-/-} MEFs. This analysis allowed us to identify 4 spots which were significantly increased and 5 spots that were decreased in NQO1^{-/-} cells compared to their normal counterparts. The proteins whose levels were increased in NQO1^{-/-} MEFs were: Tumor necrosis factor receptor-associated protein 1 (TRAP1); Chaperonin containing TCP1, subunit 6A; Glucose-6-phosphate dehydrogenase X-linked, and mitochondrial Mn-superoxide dismutase (Sod2), while the proteins whose levels were decreased in NQO1^{-/-} MEFs were procollagen-lysine, 2 oxoglutarate 5-dioxygenase 3 (PLOD3); Stress-70 protein, mitochondrial precursor (75KDa glucose-regulated protein) (GRP75); mCG12499 and Transgelin. The functions of altered proteins are related with oxidative stress, apoptosis, generation of ROS, and actin assembly. Interestingly, TRAP1 plays a relevant roles in cell-cycle progression and cellular differentiation, and its expression at high-level may be involved in anti-apoptotic effects. Increased levels of TRAP1 as a consequence of the lack of NQO1 gene expression might be related with the higher susceptibility for tumors observed in this animal model.