SYSTEMS BIOLOGY AND NICKEL CARCINOGENESIS: GLOBAL DEREGLULATION OF GENE EXPRESS-ION AND CYTOSKELETAL ALTERATIONS IN NI-TRANSFORMED 10T1/2 MOUSE EMBRYO CELLS

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Abstract: Nickel (Ni) refinery workers who inhaled Ni-containing sulfidic ore dusts and smoked cigarettes in Ni refineries contracted lung/nasal cancers. Inhalation of Ni3S2/green NiO also induced respiratory cancer in rats. We showed Ni3S2 and green and black nickel oxides were phagocytosed into and induced chromosomal aberrations, cytotoxicity, and morphological, A. I., and neoplastic transformation in C3H/10T1/2 Cl8(10T1/2) mouse embryo cells. 130 genes were differentially expressed between non-transformed and two 3-methylcholanthrene (MCA)- four Ni-transformed 10T1/2 cell lines (mRNA differential display). Ni/MCA-transformed cell lines contained a) ect-2 gene amplification/higher levels of ect-2 gene mRNA/protein; b) higher levels of calnexin mRNA/protein/Wdr1 gene mRNA; and c) no DRIP/TRAP80/β-2-centaurin-2 mRNAs. We hypothesized Ni2+ -induced 1) amplification of ect-2 gene/higher levels of rhoA-GTP led to higher levels of microtubules (MTs), and 2) silencing of β-2-centaurin-2 gene, caused higher levels/aggregation of microfilaments (MFs), and 3) silencing of the DRIP/TRAP80 gene caused aberrations in Ca2+ ion gradients, in transformed 10T1/2 cells. To test these hypotheses, we stained cells with fluorescent phalloidin to decorate MFs, separately with fluorescent antibody to α-tubulin/β-tubulin to decorate MTs, and then separately with Fluo 3AM to stain Ca2+ ions. We then examined the cells by confocal microscopy. In non-transformed 10T1/2 cells, MFs/MTs were arranged homogeneously in long thin fibers. In NiS/green NiO transformed cell lines, MFs and MTs were over-expressed and aggregated in some areas, absent in other areas, changing shapes of transformed cells, rounding them/altering their contact with extra-cellular matrix. In non-transformed cells, Ca2+ ions were found in two arrangements in non-transformed 10T1/2 cells: State I, in low density cells, with a heavy concentration of Ca2+ ions in the nucleus, and lesser amounts in the cytoplasm; and State II, in high density cells near confluence, where there were few Ca2+ ions in the nucleus, most in the cytoplasm. Non-transformed 10T1/2 cells cycled between States I and II. In Ni/MCA transformed cell lines, Ca2+ ions were predominantly cytoplasmic (State II). Our model suggests mutations/methylations in 15 genes led to differential expression of 130 total genes. We conclude Ni ions caused amplification of the ect-2 gene, and silenced expression of the β-centaurin-2 gene, leading to expression of higher steady-state levels of MTs and MFs, respectively, causing changes in cell shape, hence changes in global gene expression. Ni ions also silenced the DRIP/TRAP80 gene, which likely led to alterations in Ca2+ ion gradients in transformed cells, further altering activities of Ca2+ ion-dependent enzymes and cell physiology. These Ni ion-induced events cumulatively led to differential expression of 130 genes in transformed cell lines, altered cell shapes, and altered Ca2+ ion gradients, contributing to induction and maintenance of transformed phenotypes of transformed cell lines.

Key words: Nickel, C3H/10T1/2 Cl 8 mouse embryo cells, cell transformation, gene expression.

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