INSOLUBLE NI COMPOUNDS INDUCE CHROMOSOMAL ABERRATIONS, GENE AMPLIFICATION/GENE SILENCING, GLOBAL DISRUPTION OF GENE EXPRESSION, AND MORPHOLOGICAL/NEOPLASTIC TRANSFORMATION OF 10T1/2 MOUSE EMBRYO CELLS

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Abstract: Inhaling Ni-containing sulfidic ore dusts in Ni refineries correlates with an increased incidence of human respiratory cancer. Inhaling Ni\textsubscript{3}S\textsubscript{2} or green NiO also induces respiratory cancer in rats. Ni\textsubscript{3}S\textsubscript{2} and green NiO were phagocytosed into, and generated Ni\textsuperscript{+2} ions in, 10T1/2 mouse embryo cells, which induced morphological/A.I./neoplastic transformation in these cells. 130 genes were differentially expressed between non-transformed, and two MCA/four Ni-transformed, 10T1/2 cell lines. Ni/MCA-transformed cell lines displayed a) ect-2 gene amplification/higher levels of ect-2 mRNA/protein; higher levels of b) calnexin mRNA/protein and c) Wdr1 (stress-inducible gene) mRNA; and d) decreased/absent levels of mRNAs from DRIP/TRA80, \(\beta\)-2-centaurin, and FAD synthase genes. We hypothesized amplification of the ect-2 gene led to higher steady-state levels of rhoA-GTP, which would cause higher steady-state levels of microtubules in Ni/MCA transformed cell lines. We also hypothesized that transcriptional silencing of the \(\beta\)-2-centaurin gene in Ni-transformed cell lines led to increased steady-state levels of microfilaments (MFs) and altered their intracellular distribution, in Ni/MCA transformed cell lines. We therefore treated cells with fluorescent phalloidin, a fungal toxin that binds to MFs, and with fluorescent antibodies to \(\alpha\)-tubulin or \(\beta\)-tubulin to stain MTs, then examined cells by fluorescent confocal microscopy. MTs were distributed homogenously in non-transformed 10T1/2 cells, but present at higher levels/aggregated into clumps, in transformed cells. MFs were arranged homogeneously in long, thin fibers in non-transformed 10T1/2 cells. In three NiS-transformed/one green NiO-transformed cell lines, MFs were over-expressed/aggregated into clumps in some areas, missing in other areas. Overexpression/clumping of MFs and MTs changed shapes of transformed cells, rounding them/altering their contact with extra-cellular matrix. Loss of DRIP/TRAP80 protein in Ni/MCA transformed cell lines altered Ca\textsuperscript{+2} gradients in transformed cell lines. This would alter activities of Ca\textsuperscript{+2}-containing enzymes in transformed cell lines, leading to aberrant physiology in transformed cell lines. Our working model is that mutations/alterations in each of 15 genes occur. Each altered gene causes changes in expression of 9 additional genes, leading to differential expression of 130 genes in Ni/MCA transformed 10T1/2 cell lines, and morphological/neoplastic transformation in them.

Keywords: Nickel, 10T1/2 cells, morphological transformation, molecular biology.

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