NI^{+2} -INDUCED GLOBAL DE-REGULATION OF GENE EXPRESSION, LEADING TO MORPHOLOGICAL/ NEOPLASTIC TRANSFORMATION OF C3H/10T1/2 MOUSE EMBRYO CELLS; CR(VI)-INDUCED MORPHOLOGICAL TRANSFORMATION OF 10T1/2 CELLS AND A. I. IN HUMAN FIBROBLASTS

A Distinguished Lecture

By

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Abstract: Nickel (Ni) refinery workers who inhaled Ni-containing sulfidic ore dusts/smoked cigarettes in Ni refineries contracted lung/nasal cancers. Inhalation of Ni_3S_2/green NiO induces respiratory cancer in rats. We showed Ni_3S_2 /green/black NiOs were phagocytosed into and induced chromosomal aberrations, cytotoxicity, and morphological, A. I., and neoplastic transfor- mation in C3H/10T1/2 Cl 8 (10T1/2) mouse embryo cells. 150 genes were differentially expres- sed between non-transformed and two MCA/four NiO/NiS-transformed (Tx) 10T1/2 cell lines, shown by mRNA differential display. Ni/MCA-Tx cell lines contained a) ect-2 gene amplificat- ion/higher levels of ect-2 gene mRNA/protein; b) higher levels of calnexin mRNA/protein and Wdr1 mRNA; and c) no detectable levels of DRIP80 or β-centaurin-2 mRNAs. We hypothesized 1) Ni^{+2} -induced amplification of the ect-2 gene led to higher levels/aggregation of microtubules (MTs); 2) Ni^{+2}-induced silencing of β-2-centaurin-2 gene caused higher levels/aggregation of microfilaments (MFs); and 3) Ni^{+2}-induced silencing of DRIP80 gene altered distributions of Ca^{+2} ions, in Tx 10T1/2 cells. To test these hypotheses, we stained cells with fluorescent phalloidin to decorate MFs, with fluorescent Ab to α-tubulin to decorate MTs, with Fluo 3AM to stain Ca^{+2} ions, and with DAPI to decorate nuclei, then examined cells by confocal microscopy. In non-Tx 10T1/2 cells, MFs/MTs were arranged homogeneously in long fibers. In NiS/green NiO-Tx cell lines, MFs/MTs were over-expressed and aggregated in some areas, absent in others, changing shapes of Tx cells. In non-Tx cells, Ca^{+2} ions were found in a) State I, in low density cells, with a high concentration of nuclear Ca^{+2} ions, lower amounts in the cytoplasm; and b) in State II, in high density cells near confluence, with fewer Ca^{+2} ions in the nucleus, most in the cytoplasm. Non-Tx 10T1/2 cells cycled between States I/II. In six Ni- or MCA-Tx cell lines, Ca^{+2} ions were largely cytoplasmic (State II). Our data suggests mutations in 6 target genes and silencing of 9 target genes led to differential expression of 10 additional genes for each primary gene altered, causing differential expression of 150 genes in Tx cell lines. We conclude: 1) Ni^{+2} ions amplified ect-2 gene, leading to expression of higher steady-state levels of MTs, and silenced the β-centaur-in-2 gene, leading to expression of higher steady levels of MFs, causing changes in cell shape and gene expression, in Tx cell lines. 2) Ni^{+2} ions silenced the DRIP80 gene, which altered distribu- tions of Ca^{+2} ions in Tx 10T1/2 cell lines. 3) These Ni ion-induced events cumulatively caused differential expression of 150 genes, contributing to induction/maintenance of Tx phenotypes in Ni^{+2} /MCA-Tx cell lines. Cr(VI) compounds induced cytotoxicity and morphological/neoplastic transformation of 10T1/2 cells and cytotoxicity, A. I., and mutation in diploid human fibroblasts. Reduction of Cr(VI) by ascorbate plays a role in these processes and will be discussed.