INSOLUBLE NI COMPOUNDS AMPLIFY THE ECT-2 PROTO-ONCOGENE AND SILENCE DRIP/TRAP80 AND β-CENTAURIN-2 GENES, ALTERING GLOBAL GENE EXPRESSION, THE CYTOSKELETON, AND CA+2 GRADIENTS, INDUCING MORPHOLOGICAL/NEOPLASTIC TRANSFORMATION OF 10T1/2 MOUSE EMBRYO CELLS

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Abstract: Inhaling Ni-containing sulfidic ore dusts and smoking cigarettes in Ni refineries correlates with an increased incidence of human respiratory cancer. Inhalation of Ni3S2 or green NiO also induces respiratory cancer in rats. Ni3S2 and green NiO were phagocytosed into, and generated Ni+2 ions in, 10T1/2 mouse embryo cells. This induced morphological/AAI/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; b) increased levels of calnexin mRNA/protein and c) increased levels of Wdr1 (stress-inducible gene) mRNA; and d) absence of mRNAs from DRIP/-TRA80 and β-centaurin-2 genes. We hypothesized amplification of the ect-2 gene led to higher steady-state levels of rhoA-GTP, inducing higher steady-state levels of microtubules (MTs) in Ni/MCA transformed cell lines. We also hypothesized that transcriptional silencing of the β-centaurin-2 gene led to increased steady-state levels of microfilaments (MFs) and altered their intracellular distribution, in Ni/MCA transformed cell lines. In addition, we hypothesized silencing of the DRIP/TRAP80 gene led to alterations in Ca++ ion gradients in transformed cell lines. We treated cells with fluorescent phalloidin, a fungal toxin that binds to MFs, and with fluorescent antibodies to α-tubulin or β-tubulin to stain MTs, then examined cells by fluorescent confocal microscopy. MTs and MFs were distributed homogenously in long, thin fibers in non-transformed 10T1/2 cells, but were both present at higher levels/aggregated into clumps, in some areas and missing in other areas of transformed cells. Over-expression/clumping of MFs and MTs changed shapes of transformed cells, rounding them/altering their contact with extra-cellular matrix. Loss of DRIP/TRAP80 mRNA in Ni/MCA transformed cell lines altered Ca++ gradients in transformed cell lines. This would alter the activities of Ca++-dependent enzymes in transformed cell lines, leading to aberrant physiologies 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 approximately 130 genes in Ni/MCA transformed 10T1/2 cell lines, and induction and maintenance of morphological/neoplastic transformation.

Acknowledgements: This research was supported by grant R01 ES 03341 (PI, JRL), Core Support Grant 5P30 CA014089 to USC/Norris Cancer Center from NIH, a grant from USC Provost’s Office to support undergraduate research to JRL, and funding from the M. S. Program in Molecular Microbiology/Immunology at USC, to JRL.