Landolph et al. BMC Proceedings 2010, 4(Suppl 2):O6
http://www.biomedcentral.com/1753-6561/4/S2/O6

BMC Proceedings

ORAL PRESENTATION Open Access

Insoluble Ni compound-induced gene amplification/gene silencing causes over-expression of microtubules/microfilaments, cell shape changes, and de-regulation of global gene expression/Ca+^{2} gradients, inducing morphological/ neoplastic transformation of 10T1/2 mouse embryo cells

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From 16th International Charles Heidelberger Symposium on Cancer Research
Coimbra, Portugal. 26–28 September 2010

Inhalation of Ni-containing sulfidic ore dusts and smoking cigarettes in Ni refineries causes human respiratory cancer. Inhalation of Ni_3S_2 or green NiO induces respiratory cancer in rats. Ni_3S_2 and green NiO were phagocytosed into and induced morphological/A. I./neoplastic transformation in 10T1/2 mouse embryo cells. mRNA differential display showed that 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) absence of DRIP80 and β-centaurin-2 mRNAs. 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 stained non-transformed/transformed 10T- 1/2 cell lines with fluorescent antibody to α-tubulin/β-tubulin, to stain MTs. MTs were distributed homogeneously in long, thin fibers in non-transformed 10T1/2 cells, but present at higher levels/-aggregated in some areas/missing in other areas of transformed cells, confirming hypothesis #1. We next hypothesized 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. We stained cells with fluorescent phalloidin, a fungal toxin that binds to MFs, and demonstrated MFs were distributed homogeneously in long, thin fibers in non-transformed 10T1/2 cells, but present at higher levels/aggregated, in some areas and missing in other areas of transformed cells, confirming hypothesis #2. Over-expression/clumping of MFs/MTs rounded transformed cells, altering their contact with extra-cellular matrix. Third, we hypothesized that silencing the DRIP80 gene led to alterations in Ca^{+2} ion gradients in transformed cell lines, and confirmed this by staining cells with the Ca^{+2} fluorophore, FLUO 3AM. This would alter activities of Ca^{+2}-dependent enzymes in transformed cell lines, contributing to aberrant physiologies. Ni^{+2} ion therefore induced ect-2 gene amplification/over-expression and caused silencing of the β-centaurin-2 and DRIP80 genes, leading to over-expression of MTs/ MFs, respectively, hence cell shape changes and altered gene expression, and to aberrant Ca^{+2} ion gradients, which would cause changes in activities of Ca^{+2}-dependent enzymes. Changes in MTs, MFs, and Ca^{+2} ion gradients would de-regulate expression of 130 genes /alter Ca^{+2}-dependent enzyme activities, contributing to induction of cell transformation.

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Acknowledgements
This research was supported by grants to JRL from: R01 ES 03341 (PI, JRL), Core Grant 5P30 CA014089 to USC Cancer Center (NIH), funds from M. S. Prog. in Micro./Imm. at USC, grants from USC Provost's Office for undergraduate research.

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Published: 24 September 2010

doi:
Cite this article as: Landolph *et al.*: Insoluble Ni compound-induced gene amplification/gene silencing causes over-expression of microtubules/microfilaments, cell shape changes, and de-regulation of global gene expression/$Ca+^{2}$ gradients, inducing morphological/neoplastic transformation of 10T1/2 mouse embryo cells. *BMC Proceedings* 2010 **4**(Suppl 2):O6.