

97 Inhibition of Radiation Induced DNA-double Strand Break Repair by Heavy Metals, Arsenite and Antimony

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The inhibition of radiation-induced DNA-dsb repair by metals, arsenite and antimony, which are released from semiconductor industries, were investigated using CHO cells. Exponentially growing cells were incubated in F-12 medium with ¹⁴C-thymidine for 24 hrs, treated with NiCl₂, NaAsO₂, C₈H₄K₂O₁₂Sb₃, ZnCl₂, CdCl₂, CuCl₂ for 2 hrs, and irradiated with 40Gy γ-rays. Then the levels of DNA-dsb were determined by pulsed field gel electrophoresis immediately after irradiation and 30-minutes post-irradiation at 37°C. The rejoining of DNA-dsb was significantly inhibited by exposure to Ni, Cu, Zn, As, Sb, and Cd at concentrations of 200, 20, 20, 0.08, 0.55, and 10 mM, respectively. At these concentrations, the viability (following 2.5hr treatment) determined by trypan blue dye exclusion was more than 50% for all the chemicals suggesting that acute toxicity for these chemicals are relatively small. In contrast, plating-efficiency by longer term colony formation assay was less than 1% for all the chemicals except for As which gave 59%. These results suggest that As inhibits DNA-dsb repair at a relatively low concentration where cells sustain ability for proliferation.

98 Is the Ku70 a Target of Wortmannin, Phosphatidylinositol 3-Kinase Inhibitor?

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Wortmannin, an inhibitor of phosphatidylinositol 3-Kinase (PI 3-K) family, is a radiosensitizer of vertebrate cells, and inhibits DNA dependent protein kinase (DNA-PK) activity which mediates DNA double strand break (DSB) repair. PI 3-K family includes the DNA-PK catalytic subunit (DNA-Pkcs) gene and the Ataxia Telangiectasia (AT) gene. We investigate wortmannin sensitization to X-ray using repair proficient cells and repair deficient cell, DNA-Pkcs deficient cells (Scid), AT gene deficient cells (AT), mutants generated from the chicken B cell line DT40 (RAD54^{-/-}, KU70^{-/-} and RAD54^{-/-}/KU70^{-/-}). Either Scid or AT cells were sensitized to X-rays by wortmannin. Therefore, wortmannin on sensitization may interact with targets, other than PI 3-K family. KU70^{-/-} cells only among cells we used were not sensitized by wortmannin. The KU70 may be a target of wortmannin on sensitization.

99 Recovery of heat inactivated DNA-PK activity and HSC73

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DNA-PK was inactivated by heat treatment at 44°C for 15 min. Recovery of DNA-PK activity was observed during the incubation at 37°C following heat treatment at 44°C. When the cells were treated with cycloheximide, the recovered activity was decreased to half of untreated cells.

HSC73 was co-immunoprecipitated with Ku80 protein. The amount of co-immunoprecipitated HSC73 was increased with incubation time at 37°C after heat treatment. It was shown that HSC73 correlated the recovery of heat inactivated DNA-PK activity.