UNIVERSITY OF TORONTO
LESLIE DAN FACULTY OF PHARMACY

TOXICOLOGY GROUP TRAINEE SEMINAR PROGRAM

Wednesday, Sept. 11, 2013, 2013, 2:10–3:30 pm

Room 1210, 144 College Street

Title: Regulation of cisplatin-induced programmed cell death *in vivo*

Trainee: MAYA LATIF

Co-authors: Yanshan Cao, Kelvin Hui, Zoe Winterton-Perks, Andrew Elia

Supervisor: Dr. Jeff Henderson

Advisory committee: Drs. Micheline Piquette-Miller and Razq Hakem

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ABSTRACT:

Cisplatin [cis-$PtCl_2(NH_3)_2$] represents the canonical member of a group of platinum-based chemotherapeutic agents widely used either alone or in combination with other agents to treat a range of malignancies including sarcomas, lymphomas, germ cell tumors and several carcinoma subtypes. Use of platinum-containing compounds faces significant clinical limitation currently due to their induction of cell death in healthy proliferating tissues as well as neuro-, oto- and nephrotoxicity. The mechanism of cisplatin-mediated cell death has long been thought to be tied to nucleophilic attack of the *aquo*-substituted drug on DNA purine bases forming inter- and intra-strand crosslinks. However recent experiments in our laboratory have caused us to question this dogma. In addition the mechanism by which cisplatin induces death remain controversial, as a variety of previous studies have implicated distinct forms cell death (apoptosis, autophagy and necroptosis) in this process.

To examine the mechanism of cisplatin toxicity in greater detail, we have utilized the developing murine embryo (E12.5-15.5) as a model of rapid cellular proliferation in a variety of solid organs. *In vivo* treatment with cisplatin at levels equivalent to or below those utilized in humans clinically resulted in widespread cellular injury. In wild-type animals, this injury was characterized by p53 and gamma H2AX phosphorylation, induction of caspase-3 and caspase-7, cleavage of the caspase-dependent target poly (ADP-ribose) polymerase (PARP) and formation of TUNEL-positive DNA strand breaks. Analysis of cellular morphology by electron microscopy revealed formation of standard apoptotic bodies, indicating that under normal circumstance cisplatin promotes cell death through the induction of apoptosis. To define the mechanistic nature of this cell death, levels of cellular injury *in vivo* following cisplatin treatment were examined in mice carrying genetic modifications of apoptotic pathways. Strikingly, animals homozygous for a null mutation of caspase-3 exhibited complete inhibition of cisplatin-dependent cell death at 24 hours following treatment in a wide variety of tissues. This effect was observed despite the detection of caspase-7 activity following cisplatin treatment in both wild-type and caspase-3, demonstrating a novel functional variance between these two central executioner caspases. In addition, loss of caspase-3 may induce an alternative, normally quiescent, pathway of programmed cell death which exhibits features of necroptosis. These findings have important clinical implications toward maintenance of both normal and malignant cells in a variety of solid tissues in the presence of cisplatin.

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