UNIVERSITY OF TORONTO
LESLIE DAN FACULTY OF PHARMACY

TOXICOLOGY GROUP TRAINEE SEMINAR PROGRAM

Wednesday, May 14, 2014, 2:10–3:30 pm

Room 850, 144 College Street

Title: **Analysis of Caspase 3-dependent vs. -independent programmed cell death signalling**

Trainee: **CHESARAHMIA DOJO SOEANDY**

Supervisor: Dr. Jeffrey Henderson

ABSTRACT

Programmed cell death (PCD) is a natural process occurring in all metazoans which regulates developmental effects such as digit formation and neural target matching, as well as homeostatic control of cell number and defense against oncogenic transformation. In the central nervous system, dysregulation of PCD is a major feature of stroke and neurodegenerative diseases such as Alzheimer's, Parkinsons's and Huntington's disease. Features of several distinct forms of PCD (autophagy, necroptosis and apoptosis) have been characterized. Previous studies performed in the laboratory have suggested that at a given point in development, the same cell may exhibit both caspase 3 dependent and caspase-3 independent PCD depending upon the initiating stimulus. To determine the mechanism of this effect and decipher the molecular features regulating PCD isoform switching, I am developing a series of clonal pluripotent (ES) cell lines which differ in key PCD regulators. To rapidly generate this clonal series for analysis of network signaling interactions, I am employing the recently developed genomic re-engineering method known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). Using this technique, combinatorial mutant primary cells will be generated and tested *in vitro* and *in vivo* from lines of genetically tagged ES cells. These mutants will be exposed to various relevant PCD stimuli and signaling features determined.

TOXICOLOGY GROUP TRAINEE SEMINAR PROGRAM

Wednesday, May 14, 2014, 2:10–3:30 pm

Room 850, 144 College Street

Title: **Performance of fatty acid ethyl esters (FAEE) as biomarkers of prenatal ethanol exposure risk**

Trainee: **JOEY GARERI** MSc., PhD(c)

Supervisor: Dr. Gideon Koren

Advisors: Dr. B. Kapur, Dr. A. Taddio, Dr. K. Lançtot

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ABSTRACT

Fatty acid ethyl esters (FAEE) are non-oxidative ethanol metabolites validated as biomarkers of chronic excessive alcohol use. The performance of FAEE testing via meconium (i.e. neonatal stool) and maternal hair analysis is examined through three different thesis sub-projects:

i) *The use of FAEE meconium as a population health monitoring tool; evaluating the incidence of prenatal ethanol exposure in the entire province of Prince Edward Island.*

The use of FAEE meconium as a population-health monitoring tools was examined through population-based collection of meconium from 1307 neonates between November 2010 and November 2011 in hospitals on PEI or born to PEI mothers giving birth in Halifax, Nova Scotia. FAEE were analyzed by GC-MS and quantified using deuterated internal standards for quantitation. Of 1307 samples collected, 1271 samples were successfully analyzed. Results were positive in 3.1% of meconium samples collected within the first 24 hours after birth. It is estimated that approximately 40% of neonates exposed to heavy prenatal alcohol intake will exhibit FASD. This study suggests that a minimum of approximately 1.3% of neonates born in PEI during this one-year period will have FASD. Data collected through meconium testing showed an estimated prenatal alcohol exposure rate three-fold higher in this population than previously reported by the PEI Reproductive Care database.

ii) *Examination of sex differences in FAEE and ethyl glucuronide (EtG) hair analysis.*

Clinical studies examining performance of FAEE and ethyl glucuronide (EtG) in identifying excessive alcohol consumption have been primarily conducted in male populations. FAEE-positive hair samples (>0.50 ng/mg) from n=199 female and n = 73 male subjects for EtG. Higher FAEE/EtG concordance was observed amongst male over female subjects. Non-coloured hair demonstrated a two-fold increase in concordance over coloured hair. FAEE levels did not differ between male and female subjects, however were lower in coloured samples ($p = 0.046$). EtG was lower in female subjects ($p = 0.019$) and coloured samples ($p = 0.026$). A total of n = 111 female samples were discordant. Amongst discordant samples (EtG-negative), 26% had evidence of recent alcohol use including consultation histories (n = 20) and detectable cocaethylene (n = 9); 29% of discordant samples were coloured. False-negative risk with ethyl glucuronide analysis in females was mediated by cosmetic colouring. These findings suggest that combined analysis of FAEE and EtG is optimal when assessing a female population and an EtG cut-off of 20 pg/mg is warranted when using combined analysis.

iii) *Characteristics of FAEE production in the third-trimester equivalent fetal guinea pig.*

Pregnant guinea pigs were randomized into two groups; i) ethanol-exposed (4g/kg/day, n = 12) and ii) pair-fed controls (n = 12). On GD45, four animals from each group were randomly selected for sacrifice. Hippocampus fetal livers were excised. Hepatic CYP2E1 activity was assessed. This procedure was repeated at GD55 and GD65. The presence of [FAEE] > 0.70nmol/g had a sensitivity of 93% and specifity of 100% for chronically exposed pups with an average BEC of 76 mg/dL. A significant inverse correlation between FAEE concentration and hippocampal weight ($r_s = -0.5$) at GD55 and GD65. FAEE concentration correlated strongly with reduced microsomal 2E1 activity at GD65 ($r_s = -0.799$). These data suggest meconium FAEE may be a marker of hippocampal toxicity and suggest that interindividual variability in fetal FAEE production may mediated in part by interindividual differences in microsomal CYP2E1 activity.