



Mass Spectrometry of Buccal Mucosa -Biomarkers for Biodosimetry in Radiation Incidents

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The NYU Department of Pharmacology Mass Spectrometry Laboratory is a structural biology / nanochemistry laboratory devoted to the identification and study of tissue and body fluid biomarkers of vascular disease, tumors and putative metabolic pathways of apoptosis triggered by ionizing radiation, reactive oxygen species, ischemia and other insults. The laboratory instrumentation includes, MALDI TOF TOF, LCMS, AFM, and access to 12T FTMS, 900 MHz NMR, and 300 KEV TEM.

In the past year direct mass spectrometry identification of proteins and biomarkers of colorectal carcinoma, ischemic / stroke brain, environmental toxins, and competent in-vitro human embryos were reported by the laboratory.

Congruent with the AFFRI research and development goal of "developing methods of rapidly assessing radiation exposure to assure appropriate medical treatment", the laboratory has begun a study of the pre- and post-radiation exposure proteome of murine buccal mucosa.

Recent work has identified a transcription factor, nuclear factor KAPPA B (NF-KB) which induces the TNF- α encoding gene and activates the cyclooxygenase-2 (COX-2) pathway. At 24 hours post irradiation HIF-1 α and COX-2 protein levels were increased. In addition to its well established DNA-damage effects, ionizing radiation induces cell death, and radiation-induced activation of acid sphingomyelinases (ASMases) and the generation of ceramide. Ceramide is generated from sphingomyeline by the action of a neutral or ASMase or by de novo synthesis coordinated through the enzyme ceramidesynthase. Once generated, ceramide may serve as a second messenger molecule in signaling responses to physiologic or environmental stimuli, or it may be converted to a variety of structural or effector molecules. With a single dose of 3 Gy, there is activation of protein kinase B/AKT (PKB/AKT) signaling. Within minutes of irradiation, phosphorylation of the serine/threonin protein kinase PKB/AKT at serine-residue 473 appears. This activation of PKB/AKT contributes to inhibit glycogen synthase kinase-3beta (GSK3beta), which has a clear inhibitory role in endothelial cell survival.

This preliminary study describes the changes in murine buccal mucosa protein profiles when subjected to ionizing radiation in addition to those described above. Tissue sampling is obtained fifteen and thirty minutes post exposure. Proteins are extracted from the buccal mucosa with high pressure (Barocycler, Pressure BioSciences, South Easton, MA), the sample divided into two components. One is assigned for trypsin digestion and LCMS analysis (bottoms up proteomics), and the second one for HPLC protein separation and FTMS analysis (top-down proteomics) to identify post-translational modifications. This preclinical work heralds a clinical translation in head and neck cancer patients, for validation purposes. The long term aim is the identification of specific profiles that enable reliable associations with dose-



exposure, for biodosimetry purposes. If successful, this strategy could result in the development of a self administered diagnostic test using a buccal mucosa swab.