

Analysis of cell signaling mechanisms associated with low dose radiation exposure in a human skin model system. Feng Yang, John Miller, Ljiljana Pasa-Tolic, Marina A. Gristenko, Rui Zhao, Matthew E. Monroe, David G. Camp, Richard D. Smith and David L. Stenoien. Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA

Biological systems are exceedingly more complex than defined by the genome due to differential protein expression and degradation, altered mRNA splicing, and the presence of multiple post-translational modifications (PTMs) that regulate protein activity. Many of the responses to radiation involve PTMs on existing proteins that occur through the activation of multiple signal transduction pathways that mediate phenotypic effects. Therefore, identifying the signaling pathways and individual proteins affected by radiation should shed valuable insight into the molecular mechanisms that regulate the response to radiation. Our main objective has been to apply phosphoproteomic technologies to an intact skin model system and its individual cellular components, fibroblasts and keratinocytes, to compare and contrast radiation dose dependent signaling in cell monocultures and complex 3-dimensional tissues. Phosphoproteomic analysis of fibroblast monocultures exposed to 0, 2 or 50 cGy identified ~7117 phosphopeptides. Label free quantitation methods identified 252 proteins affected by 2 cGy exposure. To enhance quantitation an iTRAQ labeling methodology was developed specifically for highly quantitative phosphoproteomics (Yang et al, 2009). The iTRAQ isobaric labeling strategy allows for multiplexed analysis of up to eight samples in a single MS run. Application of this technology to the intact skin model system using 4 radiation doses (0, 3, 10, 200 cGy) with 4 biological replicates resulted in the identification of ~2000 phosphopeptides. Statistical analyses identified ~100 phosphopeptides showing significant changes in response to radiation treatment. In addition to highlighting signaling pathways, this data can provide insight into known radiation response pathways by identifying critical changes in phosphorylation sites that regulate specific proteins. This data can also be used to identify novel proteins that potentially play important roles in regulating responses to low doses of radiation. Further study of these radiation affected proteins may identify additional components in radiation dependent signaling networks. Finally, data from our laboratory and others indicate that many radiation responsive proteins are modified on multiple radiation dose dependent sites through the combined actions of multiple signal transduction pathways. Multiple PTMs on proteins such as histones may contribute to radiation effects on DNA repair pathways, transcription and epigenetic regulation and multisite modifications likely influence the activity of additional radiation sensitive proteins such as p53 and CHK2. To address this issue we are currently developing methods to map multiple PTMs including phosphorylation at the intact protein level.