Identification of novel ionizing radiation signaling targets in reconstituted human skin

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Our focus has been on identifying the early events that occur after low dose ionizing radiation exposure that precede and often regulate downstream events such as altered transcription, protein secretion and epigenetic regulation. Phosphorylation is one of the earliest detectible events that occurs following radiation exposure and plays important roles in multiple biological pathways including DNA damage repair, transcription, apoptosis, and cell cycle progression. Very robust changes in phosphorylation of proteins such as p53 occur at high doses of radiation however these effects are difficult to detect at low doses. While there have been numerous studies on known radiation responsive proteins such as p53 by multiple groups, there is a pressing need to take a systems biology approach to identify more of the phosphorylation events that regulate biological pathways affected by low doses of radiation. This is of critical importance for the bioinformatic analyses and computer modeling experiments carried out by our group. Moreover, changes in PTMs are often much more robust than changes in mRNA and protein levels making it more likely that these can be detected at low doses of ionizing radiation.

We recently completed a global phosphoproteomic analysis of reconstituted human skin treated with 0, 3, 10 and 200 cGy in which we identified 151 radiation affected phosphopeptides. This screen identified multiple proteins including those with potential roles in radiation responses. These include phosphorylation of promyelocytic leukemia protein (PML) at 200 cGY (3.3 fold) with possible regulation at 3 (2.4 fold) and 10 cGy (3.6 fold). Tankyrase-1 binding protein 1 (TNKS1BP1) was the most highly changed protein at 10 cGy and was also significantly affected by 200 cGy as well. Multiple proteins present in desmosomal plaques including PKP 1 and 2, desmoglein, desmoplakin, plectin 1 and multiple keratins underwent altered phosphorylation following radiation exposure suggesting that this structure is a major target of radiation induced signaling pathways. Furthermore, all of these proteins with the exception of desmoglein were affected by low doses of ionizing radiation. Desmosomes have long been known to act as anchors between adjacent cells and are extremely important for maintaining skin tissue structural integrity due to their ability to link keratin filament networks of adjacent keratinocytes. More recently, attention has been given to the idea that desmosomes can be dynamic allowing cells to respond to their external environment through desmosomal remodeling important for wound healing and differentiation or through intracellular signaling mechanisms that regulate transcription and other intracellular processes including DNA damage repair. Given that ionizing radiation can induce both tissue and DNA damage, the altered desmosomal phosphorylation observed here could play an important role wound healing and/or DNA repair processes.

We also identified altered phosphorylation of filaggrin which plays an important role in maintaining skin barrier function and skin hydration. Mutations in filaggrin are very common (~10% of the population) and cause multiple skin diseases (icthyosis vulgaris and severe eczema) as well as asthma due to its loss in lung epithelia. Profilaggrin is a very large protein with multiple repeats that are proteolyzed to generate keratin binding filaggrin monomers in the

upper layers of skin. The filaggrin monomers get further processed into natural moisturizing factors (NMFs) that maintain skin hydration. Its loss also induces chronic inflammation in skin. We found that radiation induces filaggrin phosphorylation (1.8X at 10 cGy, 4.9X at 200 cGy) at the proteolysis sites in very small linker regions located between the filaggrin monomers. Follow up experiments demonstrated that the increased phosphorylation correlates with altered filaggrin protein processing following radiation treatments.

An important issue addressed by this data is whether terminally differentiated and dividing cells in a tissue all respond to ionizing radiation. A recent study indicated that the radiation induced DNA damage response commonly observed in cell monocultures was confined mostly to the basal layers while the terminally differentiated cells in the outer layers exhibited no DNA damage response. In our data, we detected radiation induced alterations in proteins dispersed throughout the entire skin tissue including the fibroblast dermal (vimentin), the keratinocyte basal (keratins 5, 14 and 15), suprabasal (keratins 1 and 10), spinous (Keratin 2), granular (profilaggrin) and cornified (Cornifin A and B) layers and the extracellular matrix (collagens 6A3 and 17A1). This suggests that the skin as a whole responds to the radiation as judged by the altered phosphorylation status of proteins present in each layer. Moreover, different epithelial layers may have different roles to maintain skin structural integrity and genomic integrity following exposure to a tissue stress such as ionizing radiation.

While this is far from a comprehensive list of all phosphorylation sites present in the skin model, we identified many novel radiation affected proteins that are only present in skin tissue and would not have been identified in a cell culture model. We are currently enhancing this analysis by performing subcellular fractionation of the skin which serves to eliminate most of the highly abundant structural proteins such as keratins and collagens, enabling detection of more lower abundant signaling proteins. In addition, as many proteins contain a multitude of post-translational modifications, most of which are missed when doing peptide level analyses, we are utilizing a unique capability developed at PNNL to comprehensively map PTMs occurring along an intact protein. Using a novel high-throughput and high-sensitivity method for comprehensive characterization of combinatorial histone PTMs using top-down mass spectrometry we have so far identified 950 histone isoforms generated by post-translational modification. Many of these isoforms are affected by low dose radiation exposure with possible implications in DNA damage repair, transcription and epigenetic regulation.