Cell type dependent radiation induced signaling and its effect on tissue regulation

Marianne B. Sowa, Claere von Neubeck, R. Joe Robinson, Paula M. Koehler, Norman J. Karin, Xihai Wang, Katrina M. Waters and Harish Shankaran

Ionizing radiation exposure triggers a cell signaling program which includes proliferation, the DNA damage response, and tissue remodeling. The activated signaling pathways lead to the induction of both protective effects as well as adverse consequences. A fundamental question is whether signaling cascades initiated by low doses are fundamentally different than those initiated by high doses. To address this question we have applied a systems biology approach to examine the radiation induced temporal responses of an *in vitro* three dimensional (3D) human skin tissue model. Using microarray-based transcriptional profiling, our data shows that exposure to 10 cGy of X-rays is sufficient to affect gene transcription. Cell type specific analysis showed significant changes in gene expression with the levels of > 1400 genes altered in the fibroblast layer and > 400 genes regulated in the keratinocyte layer. The two cell types rarely exhibited overlapping responses at the mRNA level. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) measurements validated the microarray data in both regulation direction and value. Key pathways identified relate to cell cycle regulation, immune responses, reactive oxygen signaling, and DNA damage repair. Individual genes involved in extracellular matrix (ECM) remodeling were found to be strongly up-regulated and we have since begun looking at the expression of matrix metalloproteases (MMPs), critical regulators of the extracellular matrix. We observe a dose and time dependent induction of several MMPs at the protein level, however no corresponding changes in gene transcription were observed. We hypothesized that the initial signaling event in the MMP proteolytic cascade is through MAPK/PI3K pathway. However, the measured level of phosphorylated ERK and Akt as well as total ERK and Akt remained unaffected by low dose exposures. This finding is supported at the RNA level as neither Akt nor ERK were expressed in keratinocytes or fibroblast layers following low dose exposure. However, several genes upstream of ERK were altered and the downstream target, AP-1 (JUN), was significantly down regulated over 72 hours. Improving our understanding of these early signaling responses is necessary to develop a predictive model of the dose and dose rate dependence of low dose induced signaling.