

## **Low Dose Radiation Induced DNA Damage Signaling and Repair Responses in Human 3-Dimensional Skin Model System**

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Exposure to ionizing radiation (IR) inflicts a wide variety of lesions in the genomic DNA. Among them, DNA double strand break (DSB) is considered to be the critical lesion for most of the deleterious radiation effects including carcinogenesis. Much of our knowledge on induction and repair kinetics of DSB has come from studies in two dimensional cell culture systems. However, the damage signaling and repair responses to DSB in tissue microenvironment are largely unknown. Knowledge of tissue responses to ionizing radiation is an absolute requirement for estimating human health risks. With this objective, we have examined the time course kinetics of DSB signaling and repair responses induced by low and high doses of low LET radiation ( $\gamma$ -rays) in 3-dimensional human EpiDerm (LabTek, USA) tissue constructs. Induction and kinetics of DSB repair were monitored by analyzing some of the well-known markers for DSB such as 53BP1, MDC1 and ATM ser1981. Using 53BP1, DSB induced by as low as 0.1Gy of  $\gamma$ -rays was efficiently detected in 3D-tissues and the DSB repair was highly efficient and comparable to that observed in human primary lung and dermal fibroblasts. The efficiency of DSB repair declined with increasing doses of  $\gamma$ -rays and tissues irradiated with 5Gy showed the persistence of a few 53BP1 foci even 24 hr after irradiation. In addition to 53BP1, phosphorylation of proteins, which are targets for ATM/ATR kinases, were also detected using an antibody specific for ATM/ATR mediated phosphorylation at serine/threonine residues. Furthermore, efficient activation of phosphorylated forms of p53<sup>serine15</sup>, Chk1<sup>serine317</sup>, Chk2<sup>Thr68</sup> and DNA-PK<sup>T2609</sup> was also observed in 3-D tissue constructs demonstrating the existence of efficient DSB mediated signaling pathways in tissues. Inhibition of phosphatidylinositol 3-kinase like kinases (PIKK) by pretreatment of EpiDerm tissues with LY294002 completely abolished the 53BP1 foci formation and resulted in apoptotic death. Further studies are in progress to characterize the signal transduction pathways mediated by PIKK in tissues as a function of radiation dose.

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