Soluble/Shed factors released from skin cells following low dose irradiation exposure

David L. Springer, Colette A. Sacksteder, John H. Miller, Bobbie-Jo Webb- Robertson, Katie Lien, and Susan M. Varnum

The purpose of this work is to determine how tissues respond to low dose radiation. This includes complex interactions between cells including the release of specific proteins from cell surface. It has been demonstrated that the bystander signal can travel up to 1 mm from human skin cells that were directly hit by radiation (Belyakov, et al, 2005). This signal can be transmitted though gap junctions or through the release of soluble factors into the surrounding media by the directly hit cell. Our approach has been to irradiate 3D human skin samples with 10cGy of x-rays and collect the media at 24, 48 or 72 hrs following exposure. Using protein microarray approaches, the media was evaluated for changes in abundance against a panel of 120 inflammatory cytokines, chemokines, and growth factors. Using this approach, we determined that the concentration of a number of cytokines and chemokines are changed following exposure of the skin samples to radiation. Our results indicate that seven of the inflammatory cytokines (G-CSF, GM-CSF, IL-11, IL-17, IL-8, MCP-1 and MIP1a) are all significantly increased. Several of these cytokines function to either stimulate the differentiation of hemopoietic stem cells to mature inflammatory cells (GM-CSF and G-CSF) or function as chemo-attractants to recruit inflammatory cells (MCP-1, IL-8, and MIP1a). Additionally, we identified five chemokines that are increased significantly in the skin cell model following exposure to irradiation (ENA-78, GCP-2, MCP-2, MCP-4 and NAP-2). These chemokines all act as chemoattractants for immune cells including neutrophils. These results are consistent with other studies showing that neutrophil infiltration occurs following exposure of hemopoietic tissue with a high doses, 4 Gy, of radiation (Lorimore et al, 2001;) The other chemokines, MCP-2, MCP-4 and MIP-3alpha, chemoattract immune cells including monocytes and T-lymphocytes, are significantly increased in the cells following radiation exposure, These results indicate that inflammatory processes are activated early in the skin cell model following exposure with low doses of radiation.

Belyakov, O. V., Mitchell, S. A., Parikh, D., Randers-Pehrson, G., Marino, S. A., Amundson, S. A., Geard, C. R., and Brenner, D. J. (2005) Biological effects in unirradiated human tissue induced by radiation damage up to 1 mm away. *Proc Natl Acad Sci USA* **102**, 14203-14208.

Lorimore, S.A., Coates P.J., Scobie G.E., Milne G., Wright E.G. (2001) Inflammatory-type responses after exposure to ionizing radiation in vivo: a mechanism for radiation-induced bystander effects? Oncogene 20(48), 7085-95.