

**Author:** Oren Lerman, M.D.

**Institution:** IRPS NYU Medical Center

**Title: Low dose radiation augments endothelial progenitor cell mobilization and neovascularization in an ischemic flap.**

**Authors:** OZ Lerman, VD Thanik, MR Greives, H Le, N Seiser, R Schneider, S Formenti, PB Saadeh, JP Levine.

Laboratory of Microvascular Research, Institute of Reconstructive Plastic Surgery, NYU Medical Center, New York, NY 10016.

**Purpose:** Increasing evidence suggests that low dose radiation (XRT) can be pro-angiogenic. XRT promotes expression of HIF1 $\alpha$  as well as the CXC family of chemokines. HIF1 $\alpha$  stimulates endothelial progenitor cell (EPC) recruitment to areas of ischemia through up-regulation of the CXC chemokine SDF1. We hypothesize that low dose XRT augments neovascularization in ischemic tissue by stimulating mobilization of EPCs through HIF1 $\alpha$  and SDF1 release by endothelial cells.

**Methods:** Human umbilical vein endothelial cells were exposed to 0 or 5 Gy XRT and incubated in normoxic or hypoxic conditions. HIF1 $\alpha$  activity was measured by Western blot. Quantitative RT-PCR and ELISA measured SDF1 expression. EPC migration in response to conditioned media was measured using a trans-well assay. Apoptosis was assessed after annexinV staining on flow cytometry. Neovascularization and EPC mobilization were measured using a mouse ischemic skin flap model locally exposed to 2 or 5 Gy XRT. Blood flow was measured by laser Doppler. Vascularity was assessed by CD31 staining. EPC mobilization was measured by identification of Flk1+/Sca1+/Lin- cells in peripheral blood on flow cytometry.

**Results:** HIF1 $\alpha$  activity increased 3 fold in response to XRT. SDF1 expression after XRT peaked at 72 hrs, with an 8 fold ( $p < 0.05$ ) increase. Conditioned media harvested from irradiated endothelial cells evoked a strong chemotactic response from EPCs (275 vs 157 cells/hpf,  $p < 0.05$ ). Endothelial cell apoptosis in hypoxia decreased by 45% ( $p < 0.05$ ) 72 hrs after 5 Gy XRT. Blood flow to the ischemic flap increased 60% at 14 days after 2Gy XRT and 25% and 100% at 7 and 14 days after 5Gy XRT ( $p < 0.05$ ). Vascularity increased 20% and 60% 14 days after 2 and 5 Gy XRT ( $p < 0.05$ ). Circulating EPCs increased 57% and 73% 7 days after 2 and 5 Gy XRT ( $P < 0.05$ ).

**Conclusion:** Low dose XRT augments neovascularization in an ischemic flap. Circulating EPCs are significantly elevated in mice receiving local XRT. These results demonstrate that a single low dose of local XRT induces an angiogenic response in ischemic tissue, likely mediated by a HIF1 $\alpha$  / SDF1 dependent pathway.