

Effect of X-Ray Radiation on Adult Stem Cell Differentiation

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Statement of Purpose: In recent years, stem cell therapies have gained more and more traction and attention, especially in respect to post-cancer treatment and radiation exposure. The potential to alleviate pain and speed up the regeneration of tissue is high with stem cell therapy; however the associated cost is equally high. One of the issues involved is the high number of cells needed for injection. Many cells die before they are able to receive differentiation cues from the body that doctors inject extremely high numbers of cells to try and get a minimum number to survive. Numerous methods are known to help stem cells differentiate down a certain path *in vitro*. However, many of these techniques are difficult to translate to *in vivo* models. If a method could be devised that could be used *in vivo*, the chances of stem cell therapy success would certainly increase.

The purpose of this research is to look at the effect of radiation on adult stem cell differentiation. A variety of types of radiation is used in hospitals for treatments and imaging. While the effects of radiation on certain tissues are known, the effect on the differentiation capability of stem cells is not well understood.

Methods: Human adipose-derived stem cells (hADSCs) were expanded in standard culture conditions (5% CO₂, 37°C) in T-75 culture flasks. When the cells were approximately 50% confluent, they were taken to be irradiated. Radiation exposure was accomplished using a Phillips X-ray machine that outputs at 140 keV. Samples were exposed to a 2Gy, 4Gy or 5Gy dose then placed back in an incubator to allow growth to continue. Cells were imaged pre-exposure, immediately post-exposure and every 24 hours after exposure in order to document any proliferation or morphology changes. ALP activity was assayed 1 week after radiation exposure and the cells were fixed and stained for alpha smooth muscle actin and osteopontin.

Results: One hindrance to exposing cells to radiation is the potential to halt proliferation; however the dosage that this occurs at is not easily defined and can change between cell types. Figure 1 is a plot of cell numbers over a time period before and after exposure to gamma radiation. As expected, the control group shows a continuous increase in cell number and no halt in proliferation. After irradiation, cell proliferation appears to be dependent upon the dose. The 5Gy dose showed a gradual increase in cell number, while the 4Gy dose had an immediate decrease in cell number after exposure then

leveled off as time went by. The 2Gy dose had a large spike in cell number immediately after exposure, but then dropped back to pre-exposure levels over time.

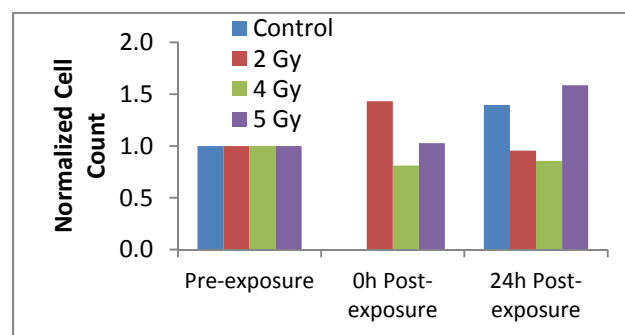


Figure 1: Cell count normalized to pre-exposure levels.

Conclusions: The effect of radiation on cell proliferation has been studied before, albeit with mixed results. Kinev et al showed that a single, low dose of radiation to endothelial colony forming cells could halt growth for as long as 72 hours. Other studies have shown evidence of increased proliferation after irradiation. All these studies use different cell types, which leads to the conclusion that different cell lines respond differently when exposed to similar levels of radiation. We can also conclude that varying doses of radiation can induce different levels of proliferation within the same cell type. It is interesting to note that despite the control group and the 5Gy group having similar cell numbers at each time point, the morphologies of each group were different (Figure 2). The 5Gy group had many more cells that had spread out as far as they could, while the control group cells mostly remained in a spindle shape.

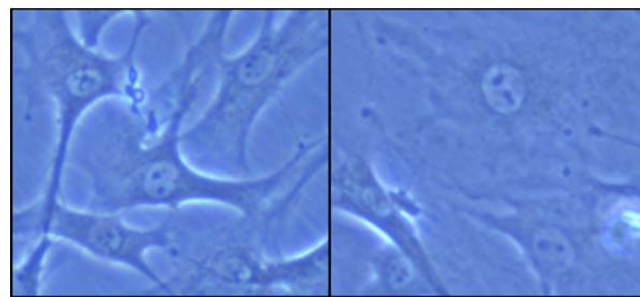


Figure 2: Left image is control (0Gy) and right image is 5Gy exposure (100x).

References:

- Kinev AV. Cancer Invest. 2013; 31:359-364.
- Jenq RR. Nat Rev Cancer. 2010; 10:213-221