An Analysis of P53 Role in DNA Damage Senescence Response to Ionizing Radiation

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Introduction

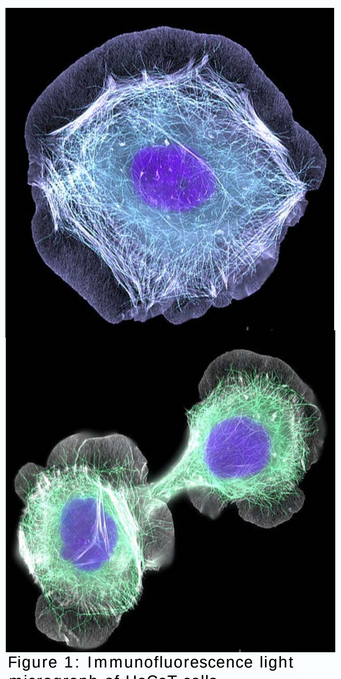

Figure 1: Immunofluorescence light micrograph of HaCaT cells
Top: Non-dividing HaCaT daughter cell
Bottom: HaCaT cell undergoing mitosis

Cosmic rays, including gamma rays and other forms of ionizing radiation, can cause major DNA damage. Energetic radiation causes double strand DNA breakage and oxidation of proteins and lipids. Much of this damaging radiation is absorbed by surface body cells (Lebel *et al*., 2011). This includes epidermal keratinocytes, which have the ability to proliferate and replace skin cells. The malignant transformation of such cells causes life-threatening skin cancers, including melanoma, basal cell cancer, and squamous cell cancer. Ionizing radiation is used in cancer treatment, where tumors are irradiated in order to kill tumors. However, as shown in this study, radiation can cause DNA damage in healthy cells surrounding the tumor, and create other cancers.

Cellular senescence is a growth arrest process that is crucial to the cell cycle, and is a response mechanism to cellular damages caused by ionizing radiation. Cell senescence limits the lifespan of mammalian cells and prevents unlimited cell division. It acts in concert with apoptotic pathways as a natural mechanism to halt transmission of damage to daughter cells by halting proliferation of abnormal cells (Sabin & Anderson, 2011).

In humans, the *TP53* gene encodes P53, a protein widely recognized as a tumor suppressor protein. It plays a major role in protection of cells from cancer and malignant transformation. It does so by regulating the cell cycle, and initiating senescence pathways once a threshold level of DNA damage is reached. This prevents proliferation of cells with DNA mutations. The HaCaT human keratinocyte cell line used in this study is an immortalized P53 mutant line.

Here, we conduct a comparison of these HaCaT cells' DNA damage response to gamma radiation versus that of normal human keratinocytes. Cells were irradiated with 1 Gray (Gy) gamma radiation, from a cesium source. Cells were then histochemically stained, identified on the basis of senescence related low pH β-galactosidase activity. Once stained, the amount of cell senescence was contrasted between irradiated cells and non-exposed wild type controls.

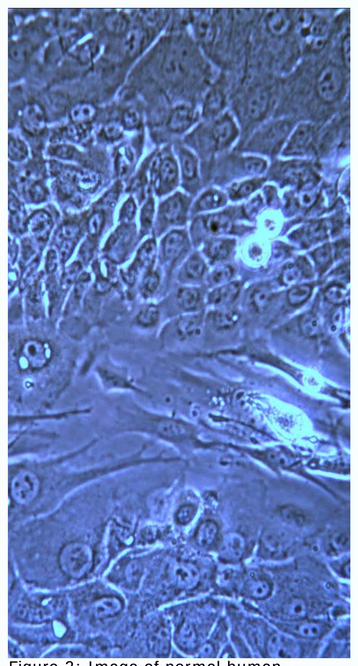

Figure 2: Image of normal human keratinocytes under a microscope

Gamma Irradiation of Keratinocytes

HaCaT and normal human keratinocyte (NHK) cells were irradiated with 1 Gy gamma radiation from a cesium source. Non-irradiated HaCaT and NHK controls were kept for comparison.

Six well plates with cells were placed on a table surface at a specified distance from the radioactive source. A Cesium-137 core was remotely elevated from an underground containment area and exposed to the cells for thirty minutes. The irradiated cells were removed from the radiation zone and taken to the lab area.

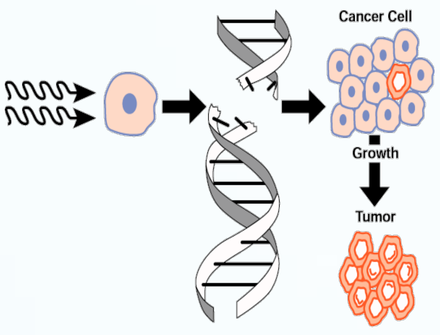

Figure 3: Development of cancer from mutation produced by ionizing radiation

Figure 3 illustrates the DNA damage effect gamma rays have on cells, creating possible malignant mutation. This damage results in the activation of cellular damage response pathways. In P53 mutant cells like the HaCaT line utilized, senescence signaling pathways are not initiated, leaving cells susceptible to cancer forming mutation (Vergel, Marin, Estevez & Carnero, 2011).

Senescence Associated β-Galactosidase Histochemistry Assay

After irradiation, cells were grown for 24 hours to allow cellular response mechanisms to take effect. Cells were then fixed and histochemically stained for low pH ß-galactosidase activity, an indicator of cellular senescence. Cells were treated and sealed, then placed into a tissue culture incubator at atmospheric CO_2 levels, due to pH sensitivity in the assay.

Once stained, senescent cells appeared blue under a light microscope. Senescent cells were easily identified. The amount of senescent cells in NHK and HaCaT cultures was evaluated, and comparisons were made between irradiated and unirradiated cells. Figure 4 shows a side by side comparison of the NHK and stained senescent cells.

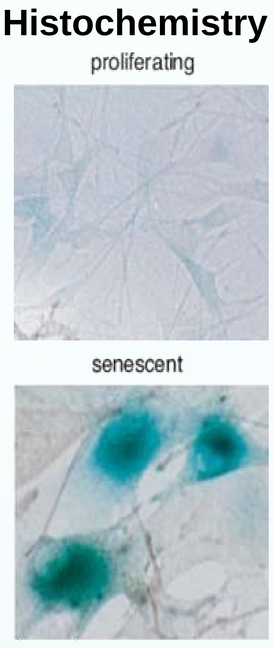

Figure 4: Normal human keratinoctyes and blue stained senescent cells

Results

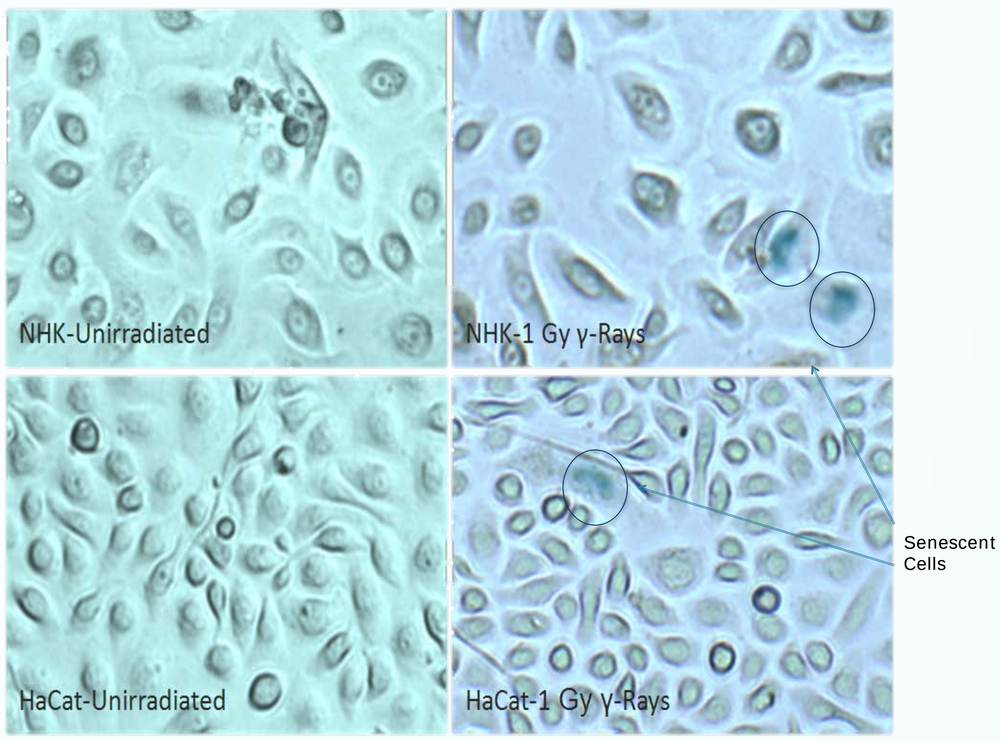

Figure 5: HaCaT and NHK senescence staining imaging

The results of one set of stainings is shown above in Figure 5. Each radiation-cell type grouping was repeated three times; results that occurred in two or more groupings were regarded as supported observations. These supported observations are imaged above.

It is clear the both the unirradiated NHK and HaCaT cells did not initiate cell senescence pathways to the same extent as the irradiated ones, presumably because ionization mediated cell damages did not occur to the same extent.

Blue staining in several cells is visible in the irradiated cell images. There are fewer senescent cells present in HaCaT P53 mutant irradiated samples than in their normal human keratinocyte counterparts.

The low dosage 1 Gy gamma irradiation was sufficient to damage cellular components and activate damage response pathways.

Discussion and Conclusion

The irradiated HaCaT and NHK samples showed considerably more senescent cells than their un-irradiated counterparts, as gamma radiation of the cells activated cell senescence pathways to a far greater extent in the radiated samples. We conclude that P53 plays an active role in cell response to ionizing radiation, mediating tumor suppressor response. This is an indicator that radiation exposure in cancer therapy can create additional tumors through DNA damage to healthy cells, as ionizing radiation is a well-known environmental threat to onset of cancer development. Wild type cells (NHK) showed much greater senescence response than the mutant HaCaT cells, confirming that P53 plays a central role in triggering senescence response after substantial DNA damage.

The focus of this research was on cellular senescence; however, cells also undergo cell death in response to ionization damages. A portion of radiation damaged cells would have become apoptotic following irradiation, which senescence staining did not capture. The whole of the damaged cells was not seen simply with senescence staining.

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