DNA Damage Pathways: Low Dose Response and Cross-Talk in TGFβ and ATM Signaling

Jennifer A. Anderson, Francis A. Cucinotta[1] and Peter O'Neill
Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford, UK
[1]NASA, Lyndon B. Johnson Space Center, Houston TX, USA

The ATM and TGFbeta signal transduction pathways are essential to cellular and tissue control responses to ionizing radiation (IR) and aberrant modifications to these pathways are extensive in cancer. We hypothesize that the ATM and TGFbeta signaling pathways are fully induced at high doses of acute low-LET radiation, whereas only partially induced at low doses. As a consequence of partial stimulation of these pathways important questions arise not only on the validity of the linear no-threshold assumption used in radiation regulations, but also on our ability to extrapolate experimental and human epidemiology data from high to low doses. The project focuses on the activating roles of Smad and ATF2 in human epithelial cells with comparison to fibroblast cells in order to elucidate how these signaling pathways alter low dose responses particularly in DNA repair, apoptosis and proliferation. Furthermore, we will investigate the role of radiation quality in the ATM and TGFbeta pathway responses by comparison of low-LET versus high-LET space radiation.

We have previously shown in rat fibroblast cells that a monoclonal TGFbeta neutralizing antibody aggregates TGFbeta signaling and that aggregation of TGFbeta signaling causes Smad1/2/3 to remain in the cytoplasm whereas the addition of exogenous TGFbeta leads to an increase in nuclear localization. These preliminary findings also indicated that radiation leads to rapid activation of latent TGFbeta present in the media as measured by an increase in Smad1/2/3 nuclear localization in irradiated cells (0.5 Gy α-particles and γ-rays).

Preliminary investigations with a normal human breast epithelial cell line (MCF10A) have shown reduced clonogenic survival following exposure to high-LET alpha-particles compared with low-LET gamma-rays. Following 2 Gy gamma-rays the number of γH2AX foci (an established marker for DNA double strand breaks) peaks 15 min following irradiation and returns to background levels at 24 h. With an equivalent dose of alpha-particles the levels of γH2AX foci peak after 30 min with approximately 35% of foci still remain at 24 h. The level of RAD51 foci (a marker of homologous recombination processes in S2/G2 phase of the cell cycle) peak 1-2 h after exposure to 2 Gy gamma-rays with foci numbers again returning to background levels after 24 h, as likewise seen with γH2AX foci. Following irradiation with alpha-particles, the levels of RAD51 foci remain high even after 24 h with no significant decrease in foci numbers over the time period examined. Preliminary results from the heavy ion experiments (^{28}Si) indicate that the percentage of MCF10A cells containing γH2AX tracks peaks 1 h after irradiation, a time longer than that seen with low-LET radiation. The levels return to background levels after 24 h. From our initial studies with MCF10A cells we have shown that the levels of γH2AX and RAD51 foci remain above background at 24 h only following high-LET alpha-particles. We are now developing the tools for carrying out low dose studies.

Using 1 Gy of ^{56}Fe or ^{28}Si heavy ions, we also examined, using immuno-histochemical assays, the nuclear translocation of Smad 1/2/3 using low (0.1 and 0.5 Gy) and high (1 Gy) doses of radiation in MCF10A and EPC2-hTERT (immortalized human esophageal keratinocytes) cells. We are investigating the recruitment and loss of Rad51, ATF2 and ATM over a 24 h period post-irradiation. Inhibition of ATM decreases the percentage of cells with γH2AX but levels again peak after 1 h and the kinetics of loss of γH2AX foci follows a similar pattern as seen in control cells.

We have also initiated preliminary experiments examining gene expression of some of the key proteins involved in the TGFbeta signal transduction pathways using RT-PCR. These early studies have shown upregulation of TGFbeta and SMAD2 in response to low-LET radiation and we will therefore expand these studies to include other proteins in the pathway as well as examining lower dose and/or effects of the radiation ionisation density.

These differences emphasise the importance of radiation quality on DNA damage repair and provide a basis for comparison when investigating the effects of TGFbeta at the DNA damage level.

This research was in part supported by the Office of Science (BER), U. S. Department of Energy, Grant number DE-SC0002296