Modulating radiation induced TGFβ and ATM signaling in the DNA damage response

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Both the ATM and TGFβ signal transduction pathways are essential for cellular and tissue control responses to ionizing radiation and aberrant modifications to these pathways are extensive in cancer. We hypothesize that the ATM and TGFβ signaling pathways are fully induced at high doses of acute low-LET radiation, whereas only partially induced at low doses. Numerous studies have linked the p38 MAPK signaling pathway with the ATM DNA damage response, and others have shown that TGFβ stimulation results in the phosphorylation of p38 MAPK. Our aim is to perturb potential crosstalk between ATM, TGFβ and p38 MAPK at the DNA damage level and investigate the resultant effects on DNA double strand break repair whilst examining whether this differs at low doses compared to high doses or depends on the quality of radiation.

We have previously shown an increase in levels of nuclear Smad 1/2/3 in MCF10A human breast epithelial cells irradiated with either high-LET heavy ions (^{56}Fe and ^{28}Si) or low-LET γ-radiation and noted that the inhibition of ATM decreases nuclear Smad 1/2/3 suggesting cross-talk between the ATM and TGFβ signal transduction pathways, independent of the radiation quality. Inhibition of ATM in MCF10A cells results in a reduction in the number of visible γH2AX foci at early times. However by stimulating MCF10A cells with TGFβ prior to ATM inhibition we are able to further reduce the appearance of γH2AX foci levels at early times (<1 h).

ATF2 is involved in transcriptional activities as well as the DNA damage response and is a common phosphorylation target of both p38 MAPK (Thr69/71 and Ser90 sites) and ATM (Ser490/498 sites). In our preliminary studies, we have utilised the specific p38 MAPK inhibitor SB203580 and have observed a decrease in γ-radiation induced γH2AX foci (using immunofluorescence), a decrease in high-LET α-particle induced ATF2 Thr69/71 phosphorylation (using immunoblotting) and a decrease in cell survival in response to γ-radiation (resazurin assay/cell survival) in MCF10A cells. We have also seen a significant reduction in ^{56}Fe, ^{28}Si and γ-radiation induced phospho-ATF2 (Ser490) and Rad51 foci following ATM inhibition (i.e. independent of radiation quality) whereas we found TGFβ stimulation decreased Rad51 protein expression (assessed by immunoblotting).

These preliminary findings indicate a role for TGFβ signaling in response to ionizing radiation induced DNA damage and crosstalk with ATM. p38 MAPK also has a function in the DNA damage response, seen as a reduction in γH2AX signaling, and this will be further explored in relation to TGFβ signaling.

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