Single-cell DNA sequencing – a potential dosimetric tool

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Background:
In 2016, Behjati et al. provided evidence that radiation-induced second tumours have distinct mutation signatures, i.e, recognizable patterns of mutations in their genomes, that can be used to distinguish them from radiation-naïve tumours. Based on this finding, we considered using DNA sequencing to search for a mutation signature in cells that are exposed to different doses and qualities of radiation immediately following irradiation, long before these cells transform into a tumour. But we know that the stochastic interactions of radiation introduce damage and mutations that are unique to each individual cell's genome and, therefore, conventional bulk-cell sequencing of a population of cells would not be able to uncover any mutation signature. Accordingly, we hypothesize that using single-cell whole-genome sequencing (ScWGS) instead will help identify such a signature. Although ScWGS is now readily available, our hypothesis has never been tested in this context, which makes this an exploratory study. We thus set out to sequence irradiated cells individually using ScWGS. To supplement our experimental work, we also performed Monte Carlo (MC) simulations of single-cell irradiation and scored immediate DNA damages to compare with our experimental findings.

Methods:
For our experiment, four identical samples of a human B-lymphoblastoid cell line were irradiated *in vitro* using 6 MV X-rays from a medical linear accelerator. They were exposed to sham irradiation (control), 0.5 Gy, 1.5 Gy and 3.0 Gy respectively at a common dose rate of 600 MU/min. Irradiated samples were incubated for 24 hrs, and then DNA was extracted from approximately 500 cells per sample and subsequently subjected to ScWGS. Our B-lymphoblastoid cell line was derived from the personal genome project, which allowed us to use their genome to establish the baseline mutations in our control and to identify radiation-induced mutations in the three irradiated samples. Copy number alterations (CNA) were identified and examined in individual sequence data from all four sample groups. Radiation-induced copy number (CN) losses were counted and compared as a function of the delivered dose.

Parallel to the experiment, we performed MC simulations. For that, we used a custom geometric nuclear DNA model of a single cell built by our group using the TOPAS-nBio framework (Montgomery et al. 2021). In our simulations, this single-cell model was stochastically exposed to secondary particles from a 250 keV x-ray beam at different dose levels: 0.0 Gy, 0.5 Gy, 1.5 Gy and 3.0 Gy, similar to our irradiation experiment. The track-structure simulation of secondary particle interactions was handled by the G4EmDNAPhysics_hybrid2and4 physics constructor (a combination of the opt2 and opt4 constructors of Geant4), and we used the TsEmDNAChemistry chemistry constructor to model all products of water radiolysis due to radiation action. We scored cluster damages introduced by radiation, specifically complex Double Strand Break (DSB) clusters, using a custom cluster scorer built previously by our group (Montgomery et al. 2021). Complex DSB clusters are of particular interest because this type of DNA damage is repaired by non-homologous end-joining and homologous recombination, both mechanisms prone to generating genomic alterations. Therefore, we counted the number of complex DSB clusters introduced with different doses of x-rays irradiating our cell model with repeated simulations.

Results:
From the sequencing experiment, we found that the number of CN losses were significantly higher in the irradiated samples compared to the sham irradiated cells. Moreover, we observed a near linear increase in the number of CN losses as a function of the radiation dose that seems to saturate at doses higher than the lethal dose to our cell line.

On the other hand, we observed a fully linear increase in the number of DSB clusters per cell as a function of radiation dose in our single-cell irradiation simulations. However, our simulations did not account for cell death. When we adjusted the output of the simulations to account for cell death based on our experimental cell-survival curve, we found that the number of DSB clusters tends to saturate at higher doses similar to what we had observed for the CN losses in the sequencing results.

Conclusion:

Our preliminary results suggest that ScWGS can be used on radiation-exposed cells to identify induced mutations and potentially quantify the radiation dose delivered. In comparison, our experimental data showed good agreement with our MC simulated results, which is promising. However, we are yet to validate our initial findings with repeated experiments. If confirmed, we posit that our strategy of examining radiation-induced DNA anomalies with single-cell whole-genome sequencing will open up new avenues for radiation biodosimetry.