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# SINGLE-CELL DNA SEQUENCING — A POTENTIAL DOSIMETRIC TOOL

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**We hypothesized that single-cell whole-genome sequencing has the potential to detect mutational differences in the genomes of the cells that are irradiated with different doses of radiation and we set out to test our hypothesis using *in silico* and *in vitro* experiments. In this manuscript, we present our findings from a Monte Carlo single-cell irradiation simulation performed in TOPAS-nBio using a custom-built geometric nuclear DNA model, which predicts a significant dose dependence of the number of cluster damages per cell as a function of radiation dose. We also present preliminary experimental results, obtained from single-cell whole-genome DNA sequencing analysis performed on cells irradiated with different doses of radiation, showing promising agreement with the simulation results.**

## INTRODUCTION

Ionizing radiation (IR)–radiation that has enough energy to ionize an atom–can introduce mutations in human cells, which may lead to carcinogenesis[1-3]. Radiation-induced carcinogenesis has been an area of active research in the field of medical biophysics for many years now[4,5], but our understanding of the biophysical mechanisms underlying it remains limited. In 2016, Behjati et al.[6] reported a potential breakthrough. They identified mutational patterns that can distinguish radiation-associated tumours from radiation-naive tumours. They observed a significant excess in the number of balanced inversion mutations and an excess in the ratio of deletion to insertion mutations in radiation-associated tumours, which they reported as a mutational signature of IR.

In light of the Behjati et al. discovery, one can imagine the possibilities for using mutational analysis of radiation-associated tumour samples to uncover the mechanisms of radiation-induced carcinogenesis. However, obtaining radiation-associated tumour samples is difficult and acquiring and identifying appropriate tumour material from biobanks may not be feasible or readily available. Therefore, in this work, we set out to develop an alternative approach to study the mutational effects of IR.

We considered the possibility of irradiating human cells *in vitro* as a means to induce genomic alterations and then analyzing the resulting genomic profiles of the exposed cells as an alternative to Behjati et al.'s approach. However, the mutations induced in an irradiated group of cells are highly heterogeneous making it difficult to interpret results for conventional bulk-cell DNA sequencing. Therefore, we proposed that single-cell DNA sequencing[7,8] might be an alternative method to elucidate the signature of radiation-induced mutations.

As a first step in testing our proposal, we investigated if single-cell whole-genome DNA sequencing of photon-irradiated human cells can uncover dose-dependent mutation effects in the genomes of these cells. In this manuscript, we present the details of our exploratory post-irradiation single-cell sequencing work and Monte Carlo[9-11] single-cell irradiation simulations.

### Monte Carlo model

Our group has previously built a geometric nuclear DNA model of a single cell in-house using the TOPAS-nBio[12] framework and made it available open-source[13]. Figure 1 shows a picture of our single-cell model in TOPAS-nBio. This simple model uses six spheres to form a nucleotide base pair that acts as the basic structural unit of the genome. Although the nucleus is constructed as a cubic grid to improve computational efficiency, our model is consistent with a human nucleus with 6.3 Gbp of DNA at a density of 13.3 Mbp $\mu m^{-3}$. Further details about our geometric model can be found in our previous publication [14].

### Complex DSB clusters

There are many different types of DNA damage that have historically been studied in similar Monte Carlo simulation studies[14-16]. These include single-strand breaks (SSBs), base lesions, double-strand breaks (DSBs), non-DSB clusters and complex DSB clusters. SSBs and base lesions are defined based on an energy deposition threshold in the DNA volume by the secondary particles via direct action, or based on spatial intersections of radiolytic species tracks with nucleotide volumes in indirect action. When two SSBs are formed on opposite strands of the DNA within 10 bp (one helical turn), it is referred to as a single DSB. A group of two or more damages within 40 bp of each other is a cluster lesion, and it is further subdivided as a non-DSB cluster and complex DSB cluster based on the absence or the presence of a DSB in the cluster, respectively [17].

### Copy Number Alterations (CNAs)

Human cells are diploid and, therefore, a normal cell is expected to have two copies of each gene in any given genomic region. Thus, a typical human cell has a mean copy number (CN) of two. When somatic amplifications or deletions in a genomic segment occur as a result of an

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abnormal event (ie exposure to DNA damaging agents, etc), the number of genomic copies at any given locus of the genome (i.e., CN) changes and this is what is referred to as a CN alteration (CNA). Genomic deletions result in a copy number loss (CN < 2) and genomic duplications cause an increase in copy number — a copy number gain (CN > 2).

## METHODS

In our Monte Carlo simulation, our 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. 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) as described by Lund et al. (2020)[18] and as adapted in our previous work[14]. Although our initially-published model was limited to simulating only direct action of radiation, in a recent work[19] (in press) we have extended our pipeline to also include indirect action to track secondary chemical species and associated damages. So for the study described here, we used the TsEmDNAChemistry chemistry constructor[20] used by Zhu et al (2020)[21] to model all products of water radiolysis due to radiation action.

In this study, we specifically analyzed complex DSB clusters and scored them using a custom cluster scorer built previously by our group[14] using the TOPAS extensions framework. Complex DSB clusters were of particular interest because they are a type of DNA damage that is difficult for a cell to repair and likely to result in a genomic alteration[22]. Therefore, we counted the number of complex DSB clusters introduced with different doses of photon exposures of our cell model with repeated simulations.

In parallel to our Monte Carlo irradiation simulations, we also exposed human B-lymphoblastoid cells (GM24385; from Corriel Institute) to four doses of 6 MV photons *in vitro*. These doses were 0.0 Gy (sham irradiation), 0.5 Gy, 1.5 Gy and 3.0 Gy consistent with the simulations. Photons were delivered using a medical linear accelerator (linac) at a dose rate of 600 MU/min as shown in Figure 2. Irradiated cells were then incubated at a temperature of 37°C with a $CO_2$ concentration of 5% for 24 hours to allow them to go through one cell cycle and attempt initial DNA damage repair. Subsequently, the DNA was extracted and amplified from ~600 individual cells in order to prepare for single-cell whole-genome DNA sequencing using the Chromium Single Cell CNV kit from 10X Genomics[23]. Prepared samples were sequenced using an Illumina NovaSeq 6000 sequencer.

To identify genomic alterations, the sequenced single-cell genomic data were analyzed in 10X Genomics' Cell Ranger DNA software using the published genome for our B-lymphoblastoid cells[24] as a custom reference genomic sequence. Our single-cell whole-genome DNA sequencing data were screened for copy number losses to simplify data analysis. The experimental cell culture assay study was designed to capture genetic alterations that occurred immediately after exposure to defined doses of radiation, where little if any time was allowed for DNA repair. Therefore, in this work, we present the mean number of copy number losses per cell measured as a function of radiation dose.

## RESULTS

Our Monte Carlo single-cell irradiation simulation results are shown in Figure 3. We observed a linear dose-dependent increase in the mean number of complex DSB clusters per cell.

Figure 4 shows the mean number of copy number losses per cell detected experimentally using the single-cell whole-genome DNA sequencing analysis in approximately 600 irradiated cells per sample. This result was obtained from just a single irradiation experiment and, as such, requires repeat assays to verify the findings. These are planned but have not yet been scheduled for logistical reasons.

## DISCUSSION

Figure 5 compares the preliminary single-cell DNA sequencing experimental results with the simulated data. Our Monte Carlo simulation of single-cell irradiation has two major limitations: (i) the model did not take into account cell death and (ii) DNA damage repair was not included in the simulation. For these reasons, the simulation result is not fully representative of a real-world single-cell irradiation scenario. We can expect that the dose-linear increase in the mean number of complex DSB clusters per cell shown in Figure 3 should plateau at the high doses at which cells die more frequently, as with the experimental result.

While our simulation used 250 keV photons for irradiation, because we adopted the model from prior work, our experiment was performed using 6 MV photons as we were limited to the use of a medical linac for irradiation, adding to the discrepancy between the two results.

The complex DSB cluster parameter from the simulations and the copy number loss from the sequencing experiment analysis are not equivalent. Therefore, we do not expect them to have exactly the same trend as a function of dose. However, we postulate that DNA damage like the complex DSBs can lead to copy number losses in the genome because they are very difficult to repair and the damage repair machinery is likely to remove the damage site[22]. Thus, we believe it is reasonable to compare the number of complex DSB clusters with the number of copy number losses per cell as a function of dose and to expect comparable trends. In this regard, Figure 5 shows a promising result in which we can see that single-cell whole-genome DNA sequencing of photon-irradiated human cells can uncover dose-dependent mutation effects in the genomes of these cells as hypothesized.

## CONCLUSIONS

Monte Carlo simulations of the irradiation of our geometric nuclear DNA model predicted that the mean number of complex DSB damages per cell should increase significantly with radiation dose. When we individually sequenced the genomes of human cells irradiated with

different doses of radiation using single-cell whole-genome DNA sequencing, we observed a comparable result in the mean number of genomic alterations per cell detected as a function of dose. Therefore, we believe that single-cell whole-genome DNA sequencing of a radiation-exposed group of cells holds potential as an alternative to the genomic analysis of radiation-associated tumours to learn about the mutational effects of IR. Since we were able to demonstrate for the first time that single-cell whole-genome DNA sequencing is sufficiently sensitive to detect dose-dependent differences in irradiated cells, we now can explore more avenues of research including, but not limited to, using single-cell sequencing as a dosimetric tool, and to potentially characterize the mutational signatures of different types of radiation such as neutrons and FLASH radiotherapy. We do caution that we are yet to validate our initial findings with repeated experiments.

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## REFERENCES

1. Ross GM. Induction of cell death by radiotherapy. Endocrine-related cancer. 41–44 (1999). DOI: 10.1677/erc.0.0060041
2. Ethel S. Gilbert. Ionising radiation and cancer risks: What have we learned from epidemiology?, International Journal of Radiation Biology, 85:6, 467-482 (2009) DOI: 10.1080/09553000902883836
3. Shah, D. J., R. K. Sachs, and D. J. Wilson. Radiation-induced cancer: a modern view. The British journal of radiology 85.1020: e1166-e1173 (2012) DOI: 10.1259/bjr/25026140.
4. Yuhas, John M, et al. Biology of Radiation Carcinogenesis. Raven Press, 1976.
5. Burns, F. J, et al. Radiation Carcinogenesis and Dna Alterations. Springer US, 1986. DOI: 10.1007/978-1-4684-5269-3.
6. Behjati, S., Gundem, G., Wedge, D. C., Roberts, N. D., Tarpey, P. S., Cooke, S. L., ... & Campbell, P. J.. Mutational signatures of ionizing radiation in second malignancies. Nature communications, 7(1), 1-8 (2016). DOI: 10.1038/ncomms12605
7. Gawad, C., Koh, W., & Quake, S. R. Single-cell genome sequencing: current state of the science. Nature Reviews Genetics, 17(3), 175-188 (2016). DOI: 10.1038/nrg.2015.16
8. Grün, D., & van Oudenaarden, A. (2015). Design and analysis of single-cell sequencing experiments. Cell, 163(4), 799-810 (2015). DOI: 10.1016/j.cell.2015.10.039
9. S. Raychaudhuri, Introduction to Monte Carlo simulation, 2008 Winter Simulation Conference, Miami, FL, USA, 91-100 (2008) DOI: 10.1109/WSC.2008.4736059.
10. Bonate, Peter L. A Brief Introduction to Monte Carlo Simulation. Clinical Pharmacokinetics, 40(1): 15–22 (2012) DOI: 10.2165/00003088-200140010-00002.
11. Harrison, Robert L. Introduction to monte carlo simulation. AIP conference proceedings. American Institute of Physics. 1204.(1) (2010) DOI: 10.1063/1.3295638.
12. Schuemann, J., McNamara, A. L., Ramos-Méndez, J., Perl, J., Held, K. D., Paganetti, H., ... & Faddegon, B. TOPAS-nBio: an extension to the TOPAS simulation toolkit for cellular and sub-cellular radiobiology. Radiation research, 191(2), 125-138 (2019). DOI: 10.1667/RR15226.1
13. Montgomery L, Lund C M, Manalad J, Landry A and Kildea J TOPAS Clustered DNA Damage GitHub repository (2021). DOI: 10.5281/zenodo.5090104
14. Montgomery, L., Lund, C. M., Landry, A., & Kildea, J. Towards the characterization of neutron carcinogenesis through direct action simulations of clustered DNA damage. Physics in Medicine & Biology, 66(20), 205011 (2021). DOI: 10.1088/1361-6560/ac2998
15. Nikjoo, H., O'neill, P., Goodhead, D. T., & Terrissol, M. Computational modelling of low-energy electron-induced DNA damage by early physical and chemical events. International journal of radiation biology, 71(5), 467-483 (1997).
16. Nikjoo, H., O'Neill, P., Wilson, W. E., & Goodhead, D. T. Computational approach for determining the spectrum of DNA damage induced by ionizing radiation. Radiation research, 156(5), 577-583 (2001). DOI: 10.1667/0033-7587(2001)156[0577:CAFDTS]2.0.CO;2
17. Sutherland, B. M., Bennett, P. V., Schenk, H., Sidorkina, O., Laval, J., Trunk, J., ... & Sutherland, J. Clustered DNA damages induced by high and low LET radiation, including heavy ions. Physica medica: PM: an international journal devoted to the applications of physics to medicine and biology: official journal of the Italian Association of Biomedical Physics (AIFB), 17, 202-204 (2001).
18. Lund, C. M., Famulari, G., Montgomery, L., & Kildea, J. A microdosimetric analysis of the interactions of mono-energetic neutrons with human tissue. Physica Medica, 73, 29-42 (2020). DOI: 10.1016/j.ejmp.2020.04.001
19. Manalad J., Montgomery, L., & Kildea, J. Simulating neutron-induced indirect DNA damage to estimate neutron carcinogenic potential from early DNA damage (in press).
20. Ramos-Méndez, J., Perl, J., Schuemann, J., McNamara, A., Paganetti, H., & Faddegon, B. Monte Carlo simulation of chemistry following radiolysis with TOPAS-nBio. Physics in Medicine & Biology, 63(10), 105014 (2018). DOI: 10.1088/1361-6560/aac04c
21. Zhu, H., McNamara, A. L., Ramos-Mendez, J., McMahon, S. J., Henthorn, N. T., Faddegon, B., ... & Schuemann, J. A parameter sensitivity study for simulating DNA damage after proton irradiation using TOPAS-nBio. Physics in Medicine & Biology, 65(8), 085015 (2020). DOI: 10.1088/1361-6560/ab7a6b
22. Asaithamby, A., Hu, B., & Chen, D. J. Unrepaired clustered DNA lesions induce chromosome breakage in human cells. Proceedings of the National Academy of Sciences, 108(20), 8293-8298 (2011). DOI: 10.1073/pnas.1016045108
23. Li R, Couturier C, Savage P, Monlong J, Bourque G, Petrecca K, et al. Abstract 2177: Sensitive single cell copy number profiling using a novel microfluidic droplet based platform. Cancer Res, 78: 2177–2177 (2018). DOI: 10.1158/1538-7445.AM2018-2177
24. Shumate, A., Zimin, A. V., Sherman, R. M., Puiu, D., Wagner, J. M., Olson, N. D., ... & Salzberg, S. L. Assembly and annotation of an Ashkenazi human reference genome. Genome biology, 21(1), 1-18 (2020). DOI: 10.1186/s13059-020-02047-7

## Figure Legends:

**Figure 1**. Our geometric nuclear DNA model developed in TOPAS-nBio; this model was previously published by Montgomery et al. (2021)[14].

**Figure 2**. A picture of our cell irradiation setup. Cells are in a flask placed on a solid water phantom on the treatment couch. The linac Gantry is at 180º.

**Figure 3**. Graph showing results from our Monte Carlo simulations. The mean number of complex DSB clusters per cell is plotted against the irradiation dose obtained from repeated simulations. Error bars are too small for visualization.

**Figure 4**. Our results from the single-cell whole-genome copy number alteration analysis of photon-irradiated cells. The mean number of copy number losses per cell for all the individual cells in a sample is plotted. Error bars show the standard uncertainty on the mean value.

**Figure 5**. The single-cell irradiation data as obtained from the single-cell whole-genome DNA sequencing (dashed) is plotted together with the Monte Carlo simulation result (dotted).