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Single-cell DNA sequencing as a means to directly examine the size and frequency of radiation-induced mutations - an exploratory study

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Disclosure Information

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I have no financial relationships to disclose.

Background

- Radiation interactions are stochastic and lead to unique mutations in each cell of a given sample.
- We hypothesize that Single-cell Whole Genome Sequencing (ScWGS) can be used to characterize the radiation-induced mutational profile of individual cells.
- Because human cells are diploid, every cell has two copies of our genome. Therefore, normal cells should have DNA with a copy number of 2.
- Any change in copy number at a location on a chromosome is referred to as a copy number variation (CNV). It is the most reliable measure of mutation with single-cell resolution using the technology we have.

Goal of this study

Our objective is to determine if ScWGS may be used to detect radiation induced damage in human cells. Also, we aim to determine the best parameter to characterize the impact of radiation on individual cells.

Experiment procedure

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1

0 Gy (Control)

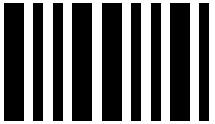
0.5 Gy

1.5 Gy

3.0 Gy

Four identical Epstein-Barr virus (EBV) transformed B-lymphoblastoid cell samples were irradiated with 6 MV X-ray radiation using a medical linear accelerator. Each sample received its dose at a common dose-rate of 600 cGy/min.

2



On average, 500 individual cells were isolated from each irradiated sample after 24hrs of incubation. Isolated cells were subsequently barcoded using a 10X Genomics Chromium controller.

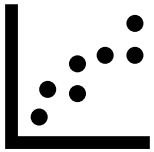
3

ScWGS of the barcoded cells was performed using the Illumina NovaSeq 6000 sequencer.

4

Sequence reads were aligned to the reference genome of our cell line using the Cell-ranger DNA software Variant-caller software were used to analyze and quantify observed genomic mutations and variants.

5



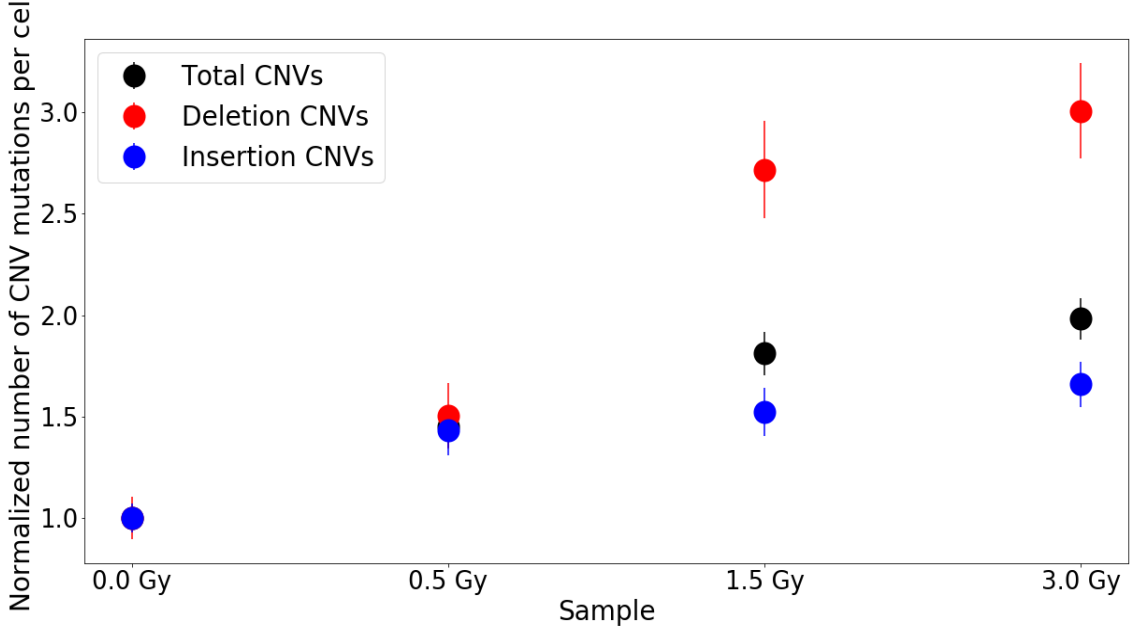
The total number of Copy Number Variations (CNVs), number of deletions CNVs and number of insertion CNVs were measured at a resolution of 20 kbp (kilo-base pairs) and plotted to identify dose-dependent mutations.

Number of CNVs vs radiation dose

Sample	**Mean number of CNVs per cell** (del. CNVs + Ins. CNVs)	**Mean number of Insertion CNVs per cell** (copy number >2)	**Mean number of Deletion CNVs per cell** (copy number <2)
0 Gy	65 ± 4	50 ± 4	16 ± 2
0.5 Gy	95 ± 7	71 ± 6	24 ± 3
1.5 Gy	118 ± 7	76 ± 6	43 ± 4
3 Gy	130 ± 7	83 ± 6	47 ± 4

Table showing the number of CNVs per cell measured for each irradiated cell sample. Deletion CNVs correspond to genomic regions with a copy number <2 and insertion CNVs correspond to a copy number >2. An unmutated genome is expected to have a copy number of 2 across all its genomic locations. Total CNVs refers to the sum of deletion and insertion CNVs. CNVs were measured at a resolution of 20 kbp. Tabulated numbers are the mean values calculated for >500 cells in each sample and the uncertainty shows the standard error on the mean value.

Increase in the number of CNVs relative to the control sample



The graph shows the relative increase in the number of CNVs per cell with radiation dose. CNVs were measured at a resolution of 20 kbp. The plotted values are the mean, and the standard error on mean for >500 cells per each sample, normalized to that of the control (0 Gy).

Our findings

- The mean number of CNVs per cell increased with radiation dose from 0 Gy to 3 Gy when each CNV corresponded to 20 kbp of DNA.
- Deletion CNVs had a higher rate of increase with radiation dose, relative to the control sample, compared to the total number of CNVs and the number of insertion CNVs.
- Number of insertion CNVs also increased, but the change was relatively smaller.
- The observed trends in our data are consistent with the increase in the number of large deletion mutations due to the introduction of radiation, reported in the literature (Behjati et al. 2016).
- If our findings are confirmed with repeated experiments, we may be able to use the number of deletion CNVs as a measure of radiation damage at the single-cell level.