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Localization Dose of Proton-irradiation Promotes DNA Damage Response

Introduction
The high-linear energy transfer (High-LET) radiation has shown to increase biological effects compared to Low-LET radiation. High-LET radiation deposits their energy locally and densely throughout their path, which allows more conformal dose delivery to the targeted cells. It is reported that biological damage from High-LET radiation is greater than the Low-LET radiation. Therefore, the purpose of this study is to prove that localized dose and its distribution can cause complex and clustered DNA damage, which is extremely difficult to repair. We observed differences in the localization doses per traversal, where DNA damage and repair are compared to the same dose in each nucleus with different number of target positions. In this study, fibrosarcoma cell lines (HT1080) cells were exposed to High-LET radiation using proton microbeam, which allow each nuclei of cell to receive the same doses of exposure. The HT1080 cells were exposed to 1 or 5 positions with the same amount of 100 or 500 protons. DNA damage induction and repair is based on γ-H2AX intensity in each cell's nuclei and cell progression in the cell cycle.

Methodology
The human fibrosarcoma cells (HT1080) were grown in specially designed microbeam dishes, with 6-μm thick polypropylene film at the bottom. Irradiation was performed using The Single Particle Irradiation System to Cell at the National Institute of Radiological Sciences (SPICE-NIRS), which delivers 3.4 MeV protons with a beam diameter of ~2 μm. Prior to radiation, cells were incubated with Hoechst 33342 for nuclei visualization and determination of the coordinates of each nucleus in irradiation region. The γH2AX immunofluorescence staining was performed to determine the DNA-DSB at 6 and 24h post-irradiation. Fluorescent images of cells were obtained using SPICE offline microscope system with two fluorescence channels, i.e. (1) cell nuclei stained with Hoechst 33342 and (2) γ-H2AX fluorescently stained with Alexa Fluor 555. Meanwhile, 1X Click-iT® EdU buffer additive Kits is used to determine cell location in S-phase of cell cycle at 6 and 24h post-irradiation.

Results
The gH2AX fluorescence intensity in nuclei is measured in relative fluorescence units (RFU). Results showed that γh2AX foci intensity decreased at 24h of post-irradiation, either HT1080 cells were exposed to 1 or 5 positions of 100 protons. Cells in both irradiation conditions were in G1-phase and the damage may still be repaired even at 24h of post-irradiation. Cells irradiated at 1 position of 500 protons showed an increase of γh2AX foci intensity, but the intensity decreased significantly at 24h post-irradiation when exposed to 5 positions of 500 protons. Regardless of whether the damaged is repaired or not, results have shown that cells were in S-phase at 24h post-irradiation. Based on these findings, the gH2AX intensity decreases at 5 positions of irradiation compared to 1 position in most post-irradiation times. This would suggest that with very high localized dose, DNA damage induced by multiple position irradiations was more effective to be repaired compared to single position irradiation. This is due to the 5 times more protons delivered and higher localized dose in the same nucleus volume, resulting in more complexed and clustered DNA damage in the cells. However, no significant increase or decrease in EdU positive cells was observed when cells were exposed to 500 protons, which we assumed that cell cycle progression was delayed and under S-phase for DNA repair.

Conclusion
Differences in dose localization lead to different levels of DNA damage. High dose localization cause severe DNA damage and are difficult to repair. This proves that localization doses induce complex and clustered DNA damage. Further studies are needed to understand the mechanism of DNA damage repair, which will clarify the involvement of inter- and intra-signal in induction of DNA damage and repair in the cells.

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