Defects in DNA Damage Response Machinery Pave the Avenue toward Radiation Quality-Specific Radiosensitivity of Cancer Cells

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Abstract

Background and aims: Technical improvements in clinical Radiation Therapy (RT) aim to maximize tumor cytotoxicity while reducing the negative impact on co-irradiated healthy tissues. This has been justified by the increasing use of particle therapy (e.g., proton therapy) worldwide. Previous work from our group and others suggested potential differences in the cellular responses to DNA damage induced by photons or protons irradiation, manifesting in shift between double-strand breaks (DSBs) repair pathways utilizations. However, the underlying mechanisms are still scarcely investigated. Here, we aim to extensively explore the underlying mechanisms involved in DSB repair of cells exposed to SOBP proton irradiation in comparison to photon-irradiated cells. Moreover, we tend to investigate the implication of homologous recombination (HR) in DSB repair process.

Methods: We utilized a panel of syngeneic ATM (an essential regulator of DSB repair by HR) and BRCA2 (a key HR factor) deficient cancer cell lines (A549, Capan1), as well as parental ATM and BRCA2 proficient cells, to elucidate the role of HR in the repair of photon or SOBP proton irradiation induced DSBs. Therefore, we are following the accumulation of γH2AX foci as a proxy indicating the formation of DSBs, long-term cellular survival, and generation of chromatid breaks. Moreover, we evaluate the HR efficiency by widely-utilized DR-GFP U2OS reporter cells following both radiation modalities.

Results: Our data demonstrate a significantly increased radiosensitivity of HR-deficient cells compared to the proficient counterpart, particularly upon SOBP proton irradiation. This increase has been indicated by survival assay results of both syngeneic cell systems, analysis of DNA repair kinetics by γH2AX foci determination in the A549 syngeneic cell model, and in experiments scoring chromatid breaks by classical cytogenetic analysis. In addition, the DR-GFP U2OS supported the DNA repair kinetics data (γH2AX and RAD51), pointing to significantly higher recruitment of HR following SOBP protons RT compared to photons RT.

Conclusions: Our data corroborate earlier observations on the increased dependency of cancer cells on DNA repair by HR following SOBP-RT using appropriate syngeneic cell systems. In addition, we reveal that HR-deficient cells display a significantly increased radiosensitivity after SOBP-RT compared to photon RT.

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References

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