Abstract

Radiation therapy (RT) is the most commonly used therapy for brain tumor patients. RT uses ionizing radiation (IR) which produces a plethora of DNA lesions in the genome of malignant cells whereas these cells are eliminated. However, IR also affects the adjacent healthy brain tissue. One of the most toxic DNA lesion produced by IR is the DNA double-strand break (DSB), and DSBs are very difficult to repair. Importantly, many studies also have shown that one of the major side effects of IR to the brain is loss of memory.

Memory is initiated and regulated by the neuronal immediate-early genes (IEGs), expression of which is controlled by physiologically-induced DSBs generated within IEGs promoters through Topoisomerase IIβ cleavage. IEGs are expressed at elevated levels immediately after neuronal activation and revert back to baseline within two hours post activation, at which time all DSBs induced by Topoisomerase IIβ are repaired. The main focus of this study is to investigate the interplay between activity- and IR-induced neuronal DNA DSBs, and to examine how RT negatively impacts upon memory. Primary cortical neuronal cells isolated from C57BL/6J mice and murine brain tissues are used. Mice and primary cortical neurons will be irradiated using γ-rays. Primary cortical cells will also be stimulated in vitro. At different times following IR exposure with and without stimulation, RNA will be extracted to establish transcript levels for Fos, Npas4 and Egr1 using qRT-PCR. Finally, in order to identify if the repair of Topoisomerase IIβ-induced DSBs is affected by IR, we will perform Topoisomerase IIβ ChIP analysis in both tissues and primary cortical neurons.

Preliminary results show that Fos is upregulated in murine cortices at 1 hour post 20 Gy γ-rays and that Fos reverts to baseline within 24 hours after exposure. Our proposed experiments will delineate some of the mechanisms by which exposure of cancer patients to IR leads to cognitive impairment. The results of this study may be significant for the development of optimized strategies of brain RT.

Materials and Methods

Cells/Animals and Treatments
Cells: primary cortical murine neuronal cells
Mice: C57BL/6J
Treatments: naïve, sham-IR and 2 Gy γ-rays

Methods
1. Expression of IEGs: RT-qPCR
2. Repair of Topo IIβ-induced DSBs using ChIP analysis (with and without IR)

Experimental Design

Expression of IEGs 30 minutes after induction

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Fos</th>
<th>Npas4</th>
<th>Egr1</th>
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- Peak expression of all IEGs 30 minutes after stimulation
- At 2 h after stimulation expression goes back to normal
- Topo IIβ-induced DSBs are repaired within 2 h after stimulation via NHEJ

Future Experiments

- Perform Topo IIβ ChIP - Measure the repair of physiologically-induced DSBs
- Perform RT-qPCR for the remaining IEGs (Npas4, Egr1) – Measure “memory formation”
- Include non-irradiated and irradiated neuronal cells.
- Include naïve and mice stimulated or treated with sham-IR or IR

References