

Role of NADPH Oxidase in Low-dose Radiation-induced Neurovascular Remodeling in Mouse Hippocampus

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PURPOSE AND BACKGROUND

Our knowledge about the specific cellular mechanisms of low-dose ionizing radiation-induced damage in hippocampus and subsequent pathological and functional consequences are very limited. The development of radiation-induced effects involves an acute and chronic oxidative stress. Despite the established link between reactive oxygen species (ROS) overproduction and pathological neurodegeneration, the source of ROS and the cell type involved remain unknown. The purpose of this study was to investigate whether the superoxide-producing enzyme NADPH oxidase is involved in alterations of neurovascular remodeling induced by low-dose proton radiation.

METHODS

Whole-body of C57BL/6 mice (n=6/group) were exposed to single dose of 0.5 Gy of proton radiation. Brains were harvested, and hippocampi were isolated from subset of the groups (n=3) for analyses at 4 and 24 hours thereafter for gene analysis. Changes in expression of genes involved in cellular functions of endothelial cell biology and genes implicated in oxidative stress were examined by quantitative RT-PCR at 4 hours post-irradiation. Immunohistostaining (IHC) for an oxidative damage marker on brain sections was also performed using antibody against Nox2, a critical component of NADPH oxidase. Changes in matrix metalloproteinase-2 (MMP-2) and intercellular adhesion molecule (ICAM) levels, which are responsible for extracellular matrix (ECM) remodeling, were evaluated in hippocampus at 24 hours post-irradiation using Western blotting assay.

RESULTS

We found that many genes which play critical roles in mediating neurovascular oxidative stress were significantly changed ($p < 0.05$) in expression following low-dose radiation at 0.5Gy compared to controls. Several genes responsible for regulating the production of ROS were significantly up-regulated compared to controls, including: *Noxa1*, *Nox1*, *Nox2*, *Rag2*, *IL-22*, and *Hsp70*. These findings indicate that ionizing radiation can activate individual components of the NADPH oxidase complex which is associated with oxidative stress. Our IHC study showed that Nox2 was highly induced in the endothelial cells of irradiated mouse hippocampus when compared to that of controls. This observation provides evidence that Nox2 containing NADPH oxidase contributes to radiation-induced oxidative stress. Among the 84 genes associated with endothelial cell biology, the expression of 26 genes was significantly different in the hippocampus after 0.5Gy compared to 0 Gy ($p < 0.05$). 7 genes responsible for regulating oxidative stress following endothelial cell injury in hippocampus were significantly regulated compared to cortex. For an ECM profile, 8 genes participating in ECM remodeling were also greatly altered, including: *Ace*, *Adam17*, *Agt*, *Flt1*, *Itga5*, *Mmp2*, *Mmp9*, and *Vwf*. At 24 hours following proton irradiation at 0.5Gy, protein level of MMP-2 and ICAM in the brain were significantly increased compared to controls ($p < 0.05$). The data suggest that low-dose radiation induced ECM remodeling in the brain.

CONCLUSIONS

This study demonstrates that ionizing radiation induces gene and cellular responses in mouse brain at a dose as low as 0.5 Gy. Diverse changes in gene expression profiles and proteins of oxidative stress and ECM remodeling in hippocampal endothelial cells in response to radiation exposure supports our hypothesis that NADPH oxidase is a critical source of the neurovascular oxidative stress following low-dose ionizing radiation that mediates brain vascular and tissue remodeling.