O-linked N-acetylglucosamine (O-GlcNAc) glycosylation and Neuroprotection from Radiation Necrosis in Gliomas

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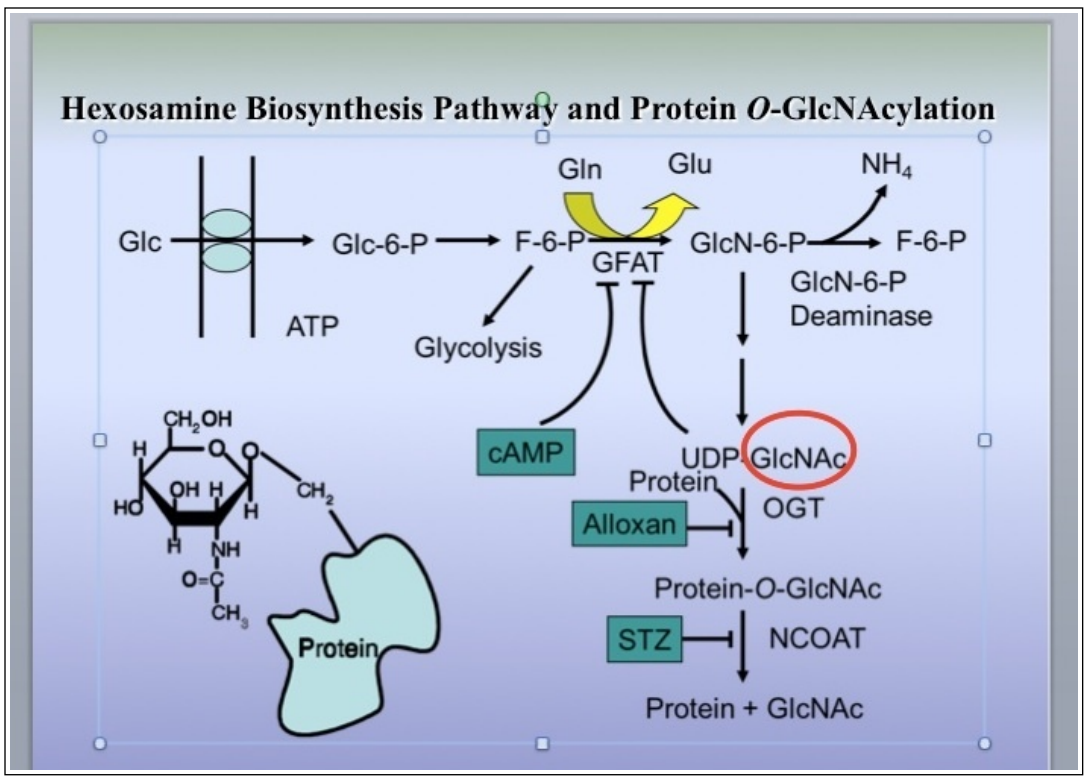
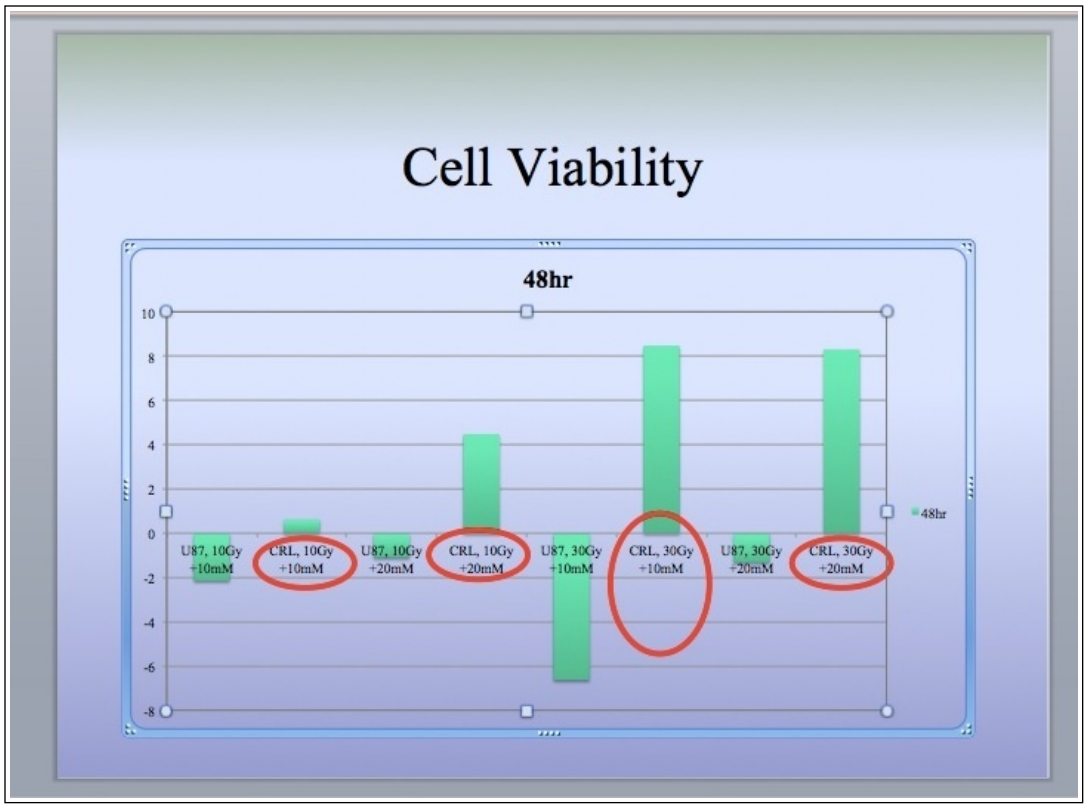
Introduction

Previous evidence indicates that the modification of proteins by O-linked N-acetylglucosamine (O-GlcNAc) is closely linked to multiple neuropathologies5,4,3. More importantly, two enzymes responsible for O-GlcNAc cycling are abundant in the brain1. Lastly, recent evidence shows glucosamine and O-GlcNAc's neuroprotective effect via suppression of inflammation in reperfusion injury2. This implicates O-Glc-NAc and glucose metabolism as potential protector against radiation necrosis in glioma therapy. With this effort in mind, we investigate the neuroprotective effect of glucosamine (GlcN).

Methods

Prior to different doses of x-ray radiation, we pharmacologically increased O-GlcNAcylation via application of varying GlcN to gliomas and primary astrocytes. We used western blotting to detect and quantify radiation necrosis markers. We conducted cell viability assays to confirm O-GlcNAc's protective role in primary astrocytes. To conduct in vivo experiments, we injected tumor cells into mice and applied GlcN intra-peritoneally prior to radiation. Using immunohistochemistry, we then analyzed brain sections for radiation necrosis and conducted protein quantification.





Results

Application of glucosamine shows decrease in phospho-H2AX (histone 2 AX), radiation necrosis marker in primary astrocytes, but not in glioma cells. Increasing dose of radiation leads to greater decrease in radiation necrosis marker with the presence of glucosamine. There is dose-response relationship. Furthermore, cell viability results confirm that primary astrocytes' viability after undergoing radiation is increased with application of glucosamine compared to tumor cells. In addition, primary astrocytes show greater O-GlcNacylation of proteins compared to glial tumor cells after undergoing radiation, which sheds light on the underlying mechanism. These results are confirmed in both in vitro and in vivo models.

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