Inhibition of hypoxia inducible factor 1-alpha causes oxygenindependent cytotoxicity and induces p53 independent apoptosis in glioblastoma cells.

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Abstract

PURPOSE: Hypoxia, which activates the hypoxia inducible factor-1 alpha (HIF-1alpha) pathway, is a common feature in malignant gliomas and has been linked with tumor cell survival and therapy resistance. In this study, we examined the effect of antisense inhibition of HIF-1alpha on the survival, apoptosis and responses to chemotherapy in U-87 malignant glioma cells. MATERIALS AND METHODS: Hypoxia (1% oxygen) was achieved in a tri-gas incubator with intermittent N(2) gas flushing or in a gas tight-module sealed with 94% N(2), 1% O(2) and balance CO(2). HIF-1alpha inhibition was achieved with antisense phosphorothioate oligodeoxynucleotide (AS-HIF ODN), delivered using cytofectin GSV3815. HIF-1alpha expression level was monitored by a hypoxia-responsive luciferase reporter assay and verified by northern blot and immunoblot analyses. Cell viability was quantified by a colorimetric microtiter plate MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] assay. Apoptotic cell death was detected with a colorimetric caspase-3 assay, as well as using terminal transferase-catalyzed in situ end labeling (TUNEL) staining. RESULTS: Antisense HIF-1alpha phosphorothioate oligodeoxynucleotide (AS-HIF ODN) treatment suppressed HIF-1alpha expression by up to 80% under both normoxic and hypoxic conditions as measured by a hypoxia-responsive reporter assay and confirmed by northern and western blot analyses. Antisense knockdown of HIF-1alpha resulted in significant reduction in U-87 cells survival and an acceleration of apoptosis, which did not involve p53 transactivation. Pretreatment of cells with Z-Val-Ala-Asp (-OCH(3))-fluoromethylketone (Z-VAD), a broad-spectrum caspase inhibitor largely eliminated this effect of AS-HIF. Caspase-3 specific activity was markedly induced 3 days after AS-HIF treatment when increased cell death was also noted. Transient overexpression of HIF-1alpha in U-87 cells neutralized apoptosis-inducing effect of AS-HIF. AS-HIF treatment did not affect viability of primary astrocytes and was selectively more toxic to U-87 glioma cells than normal human fibroblasts. The

HIF-1alpha antisense treatment exerted an oxygen-independent, and additive but not synergistic effect to the cytotoxicity of cisplatin, etoposide, and vincristine. CONCLUSIONS: These results together indicate that suppression of HIF-1alpha-expression may be a promising strategy that is selective for reducing the survival and facilitating chemotherapeutic efficacy of malignant glioma.