Protein Phosphatase 2A Mediates Dormancy of Hypoxic Glioblastoma Multiforme-derived Tumor Stem-like Cells

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Introduction

The hypoxic microenvironment of glioblastoma multiforme (GBM) is thought to increase resistance to cancer therapies. Recent evidence suggests that hypoxia induces activity of protein phosphatase 2A (PP2A), a regulator of cell cycle and apoptosis. The effects of PP2A activity on tumor cell proliferation and survival in GBM have not been studied.

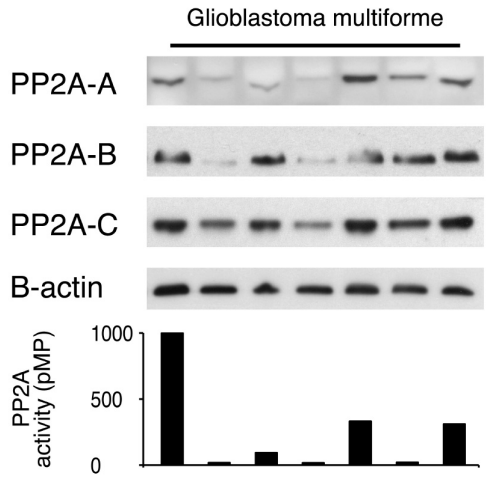
Methods

65 high-grade astrocytomas were evaluated for PP2A, HIF-1a, and Cyclin G2 protein expression as well as for PP2A activity. PP2A activity was modulated by okadaic acid in hypoxic GBM-derived tumor stem-like cells (TSCs). Effects of PP2A activity cell survival were assessed using flow cytometry.

Results

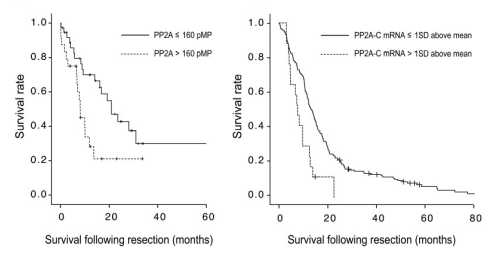
High PP2A activity was detected in hypoxic GBM specimens and was associated with poor clinical prognosis. Moderate hypoxia induced PP2A activity in TSCs in vitro. PP2A activity was mirrored by increased expression of cyclin G2, an unconventional cyclin that mediates G1/S phase cell cycle arrest. Cyclin G2 was detected as a binding partner of PP2A in TSCs. Inhibition of PP2A activity by okadaic acid or shRNA partially reversed cell cycle arrest typically seen in hypoxic TSCs. Inhibition of PP2A in hypoxic cells increased PLK expression, AKT phosphorylation and was associated with increased cell death.

PP2A expression in GBM



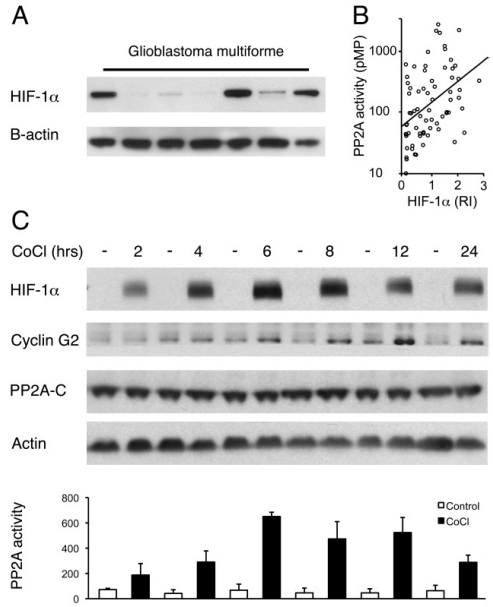
PP2A Protein expression levels are significantly correlated with PP2A activity in GBM tissue samples

PP2A predicts poor survival



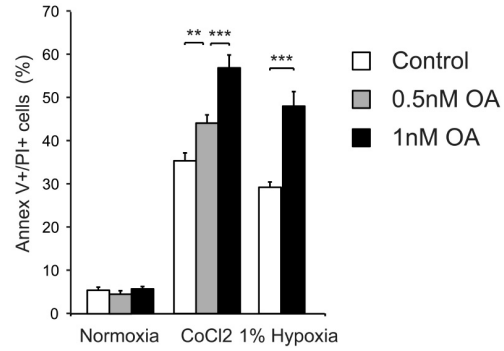
Both, High PP2A activity (A) and high PP2A-C mRNA expression (cancer genome atlas, B) predict poor survival in patients with GBM

Hypoxia induces PP2A activity



Examples of HIF-1 expression in GBM specimens (A). HIF-1 protein expression levels correlate significantly with PP2A activity (P = 0.002, B). CoCl2 exposure gives rise to HIF-1 protein expression in TSC, while TSCs grown in standard conditions lack detectable expression (C). Increased cyclin G2 levels are detected in TSCs six hours following exposure to CoCl2, while expression of PP2A-C subunit remains stable throughout the experiment. Bar graph depicts marked increase of PP2A activity following exposure of TSCs to CoCl2.

PP2A protects hypoxic TSCs



PP2A inhibition by okadaic acid (OA) increases the amount of Annexin V+/PI+ TSCs during hypoxic conditions.

Conclusions

Our results demonstrate that PP2A activity mediates dormancy of hypoxic GBM-derived TSCs. Pharmacological inhibition of PP2A decreases TSC survival in hypoxic conditions and may be a possible target for cancer therapy.

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the role of protein phosphatases in tumor cell survival, 2) Identify the impact of hypoxia on the behavior of GBM-derived stem cells, 3) Discuss strategies to target hypoxic tumor cells with cytotoxic agents.

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