Glioblastoma (GBM) is, in essence, an incurable cancer, with most patients surviving 12-15 months following initial diagnosis. Previous studies identified Glycerogen Synthase Kinase-3 (GSK-3) as a new therapeutic target in GBM. Because GSK-3 is a key regulator of NF-κB-mediated survival and chemoresistance in cancer cells, we hypothesized that the inhibition of GSK-3 may overcome NF-κB-mediated chemoresistance to conventional drugs in human GBM. Using SDS-PAGE imaging of lysates and western blotting, we found that: 1) NF-κB is constitutively active in orthotopic GBM patient-derived xenograft (PDOX) tumors and orthotopic GBM cell lines expressing an NF-κB luciferase reporter, and 2) A single intravenous injection of our novel GSK-3 inhibitor 9-NIG-41 significantly inhibited 9-NIG-41 transcriptional activity in orthotopic GBM PDOX tumors. Using two different GFB PDOX tumor models (GBM7 and GBM10), enabled tumors bioluminescence imaging through luciferase reporter transduction; we evaluated the antitumor activity of 9-NIG-41 alone as well as in combination with irinotecan, CCNU and temozolomide. Our in vivo results revealed that treatment with a combination of 9-NIG-41 and CCNU led to a complete regression of orthotopic GBM and GBM12 tumors. Histological evaluation of mouse brain confirmed the abatement of cancer cells in mice treated with 9-NIG-41+CCNU. We provide a rationale to advance 9-NIG-41 for clinical evaluation in treating GBM, especially when combined with CCNU cytoprotic therapy.

Introduction

Malignant brain tumors represent one of the most devastating and incurable cancers. Treatment with conventional chemotherapeutic drugs has had little impact on glioblastoma (GBM) progression. Thus, GBM represents a significant unmet medical need and the identification of new therapeutic agents is urgently needed for the effective treatment of GBM to improve clinical outcomes. The molecular analysis of brain tumor biopsies identified that NF-κB and its target genes are overexpressed in GBM and astrocytoma tumors compared to normal brain tissue (1, 2). In addition, a positive correlation between NF-κB activation and poor GBM prognosis was reported (3). Aberrant NF-κB activity was found critical for GBM massive phenotypic changes and resistance to cytotoxic agents in GBM (1, 4). Thus, targeting components of NF-κB signaling activation might represent a useful therapeutic approach to overcome malignant brain cancer growth and chemoresistance. Glycerogen Synthase Kinase-3 (GSK-3), a serine/threonine protein kinase, is an essential positive regulator of NF-κB transcriptional activity (5). Our previous study demonstrated that the inhibition of GSK-3 reduces cancer cell survival via suppression of the NF-κB-mediated expression of pro-survival genes (6). Moreover, we have demonstrated that AR-A014418, a toolbox GSK-3 inhibitor, enhanced the antitumor effect of docetaxel and synergistically decreased the viability of renal cancer cells (10). Similarly, AR-A014418 was shown to sensitize pancreatic cancer cells to gemcitabine (12). Finally, a number of published studies identified GSK-3 as a new therapeutic target in human GBM (13-15).

Abstract

Malignant brain tumors represent one of the most devastating and incurable cancers. Treatment with conventional chemotherapeutic drugs has had little impact on glioblastoma (GBM) progression. Thus, GBM represents a significant unmet medical need and the identification of new therapeutic agents is urgently needed for the effective treatment of GBM to improve clinical outcomes. The molecular analysis of brain tumor biopsies identified that NF-κB and its target genes are overexpressed in GBM and astrocytoma tumors compared to normal brain tissue (1, 2). In addition, a positive correlation between NF-κB activation and poor GBM prognosis was reported (3). Aberrant NF-κB activity was found critical for GBM massive phenotypic changes and resistance to cytotoxic agents in GBM (1, 4). Thus, targeting components of NF-κB signaling activation might represent a useful therapeutic approach to overcome malignant brain cancer growth and chemoresistance. Glycerogen Synthase Kinase-3 (GSK-3), a serine/threonine protein kinase, is an essential positive regulator of NF-κB transcriptional activity (5). Our previous study demonstrated that the inhibition of GSK-3 reduces cancer cell survival via suppression of the NF-κB-mediated expression of pro-survival genes (6). Moreover, we have demonstrated that AR-A014418, a toolbox GSK-3 inhibitor, enhanced the antitumor effect of docetaxel and synergistically decreased the viability of renal cancer cells (10). Similarly, AR-A014418 was shown to sensitize pancreatic cancer cells to gemcitabine (12). Finally, a number of published studies identified GSK-3 as a new therapeutic target in human GBM (13-15).

Results (1)

We developed subcutaneous (subQ) and orthotopic GBM PDOX tumor models using freshly dissociated human GBM tumor specimens from brain cancer patients. GBM PDOX models preserve the key features and cellular diversity of human GBM tumors. A small piece of GBM PDOX tumor was dissociated and the tumor cell suspension was transplanted overnight with lentivector encoding fluorescent (Tomato) and bioluminescent (Luc2) genes ex vivo.

Figure 1: Hypothesis: inhibition of GSK-3α, a positive regulator of NF-κB activity, overcomes NF-κB-mediated chemoresistance and thereby potentiates the effect of conventional chemotherapeutics in human GBM.

Figure 3: Development of orthotopic GBM PDOX xenograft bioluminescence imaging platform.

Figure 4: Characterization of orthotopic GBM PDOX xenograft bioluminescence imaging platform. A, B: Photographic images of biodistribution of luciferase reporter following injection of orthotopic GBM PDOX tumor (PCF) cells expressing luciferase reporter or luciferase reporter expression in orthotopic GBM PDOX tumor (PCF).

Results (2)

We tested whether inhibition of GSK-3α, a positive regulator of NF-κB activity, using our novel and proprietary compound 9-NIG-41, overcomes NF-κB-mediated chemoresistance to conventional chemotherapeutic drugs in human GBM. Our goal was to first a combination of 9-NIG-41 and cytotoxic chemotherapy to achieve the repression of GBM tumor growth. To determine the optimal initial dosage, schedule and combination of 9-NIG-41 with irinotecan, temozolomide and CCNU, we performed each experiment in a panel of xenograft cell lines (Fig. 4).

Figure 5: Treatment with CCNU+9-NIG-41 causes complete regression of orthotopic GBM. A: Kaplan-Meier survival analysis (progression-free survival) of orthotopic human GBM PDOX tumors. Animals were divided into a control and 9-NIG-41+CCNU treatment group. Tumors were measured weekly until animals were sacrificed when tumors reached 1 cm. B: Representative pictures of orthotopic GBM PDOX tumor (PCF) xenografts from animal sacrificed after progression-free survival measure. C: Graph plotting the mean tumor volume from each group of animals (A11: P = 0.03; A21: P = 0.03).

Figure 6: Representative bioluminescence imaging of brain luciferase reporter expression following treatment with the combination of 9-NIG-41 and CCNU in orthotopic GBM PDOX tumors (Fig. 5-7).

Results (3)

Conclusions

Clinically, initial GBM response to chemotherapy has two scenarios: 1) refractory tumor with no response, or 2) partial response with arrest or growth arrest of tumor. Our results demonstrate that GSK-3 inhibitor 9-NIG-41 (alone or in combination with CCNU) resulted in significant growth suppression of GBM PDOX tumors (3-4) and identified a new GBM in vivo model to test new drug combinations of CCNU and 9-NIG-41. Our results validate the hypothesis that GSK-3 inhibitor 9-NIG-41 is a potential therapeutic agent in GBM, and further studies are needed to assess its clinical effectiveness in GBM.

References