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Inhibition of polo-like kinase 1 in glioblastoma multiforme induces mitotic catastrophe and enhances radiosensitisation

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Abstract Glioblastoma multiforme (GBM) is the most common primary brain tumour in the United States of America (USA) with a median survival of approximately 14 months. Low survival rates are attributable to the aggressiveness of GBM and a lack of understanding of the molecular mechanisms underlying GBM. The disruption of signalling pathways regulated either directly or indirectly by protein kinases is frequently observed in cancer cells and thus the development of inhibitors of specific kinases has become a major focus of drug discovery in oncology. To identify protein kinases required for the survival of GBM we performed a siRNA-based RNAi screen focused on the human kinome in GBM. Inhibition of the polo-like kinase 1 (*PLK1*) induced a reduction in the viability in two different GBM cell lines. To assess the potential of inhibiting PLK1 as a treatment strategy for GBM we examined the effects of a small molecule inhibitor of PLK1, GSK461364A, on the growth of GBM cells. PLK1 inhibition arrested cells in the mitotic phase of the cell cycle and induced cell kill by mitotic catastrophe. GBM engrafts treated with GSK461364A showed statistically significant inhibition of tumour growth. Further, exposure of different GBM cells to RNAi or GSK461364A prior to radiation resulted in an increase in their radiosensitivity with dose enhancement factor ranging from 1.40 to 1.53 with no effect on normal cells. As a measure of DNA double strand breaks, γH2AX levels were significantly higher in the combined modality as compared to the individual treatments. This study suggests that PLK1 is an important therapeutic target for GBM and can enhance radiosensitivity in GBM.
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1. Introduction

Glioblastoma multiforme (GBM) is the most common type of malignant primary brain tumour.[1] The standard of care for newly diagnosed GBM is maximal surgical resection followed by radiation and chemotherapy. The median progression-free and overall survival times for patients treated with the current standard chemo-radiotherapy are approximately 7 and 15 months, respectively.[2,3] On the other hand there is no standard chemotherapy for recurrent or progressive GBM. Hence, identification of novel molecularly targeted therapies for GBM would be of great interest.

Cellular kinases are a family of proteins critical to all signal transduction pathways involved in cell proliferation, growth, survival, adhesion, motility and differentiation.[4] Deregulation of kinase-mediated signal transduction is implied in GBM tumourigenesis [reviewed in Refs. 5, 6]. Therefore, the analysis of all kinases (the kinome) may yield information on aberrant cell signalling pathways in GBM. Several strategies can be used to study the kinome, including Western blot analysis, Enzyme-linked immunosorbent assay (ELISA), mass spectrometry and phosphor-proteome identification by tyrosine specific antibodies. However, these methods are either not amenable to high-throughput analysis or are labour intensive. An alternative strategy is the application of loss of function (LOF) RNA interference (RNAi) screens using chemically synthesised siRNAs or plasmid-encoded short hairpin RNAs (shRNAs). This unbiased functional genomic approach has the potential to identify tumour cell specific signalling pathways and thus novel drug targets.[7–10]

In this study we conducted a siRNA-based screen focused on the human kinome in the well-established GBM cell line U87-MG and identified PLK1 as the most robust putative target, which can radiosensitise GBM tumours to radiation therapy.

2. Materials and methods

2.1. Cell lines and drug treatment

The LN18 and U87-MG (ATCC, (Manassas, VA) and the U251 (National Cancer Institute Frederick Tumour Repository) human GBM cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS), and maintained at 37 °C, 5% CO_2. GBAM1, GBM stem cells were established from patient resections as previously described[11] and grown in DMEM/F12 (Invitrogen) containing B27 supplement (1x, Invitrogen), basic fibroblast growth factor and epidermal growth factor (50 ng/ml each, Sigma–Aldrich, St. Louis, MO). MRC9 (normal lung fibroblasts) were obtained from ATCC and maintained in minimum essential medium supplemented with 10% FBS, glutamine, sodium pyruvate and non-essential amino acids. GBM stem cells and normal cells were used between passages 3 and 7. GSK461364A was purchased from MedChem Express (MedChem Express, China), was reconstituted in dimethyl sulfoxide and stored at −80 °C. Cells were plated 24 h prior to drug treatment and were treated with GSK461364A at the concentrations indicated in each experiment.

2.2. siRNA based analysis

All siRNAs were arrayed from the Human Druggable Genome siRNA Set Version 2.0 (Qiagen Inc., Germantown, MD). RNAi screens were conducted using synthetic siRNAs corresponding to 691 genes annotated at purchase as associated with kinase activity (Table S1). A detailed protocol is given in the Supplementary methods.

The sequences for the PLK1 siRNAs used in this study are as follows siPLK1.2: 5′ CAACGGCAGCG TGCAGATCAA 3′ (SI00071624), siPLK1.3: 5′ CACC ATATGAATTGTACAGAA 3′ (S100071631), siPLK1.4: 5′ CCCGAGGTGCTGAGCAAGAAA 3′ (S100071638) and siPLK1.7: 5′ CGCGGGCAAGAT TGTGCCTAA 3′ (SI02223844) (Qiagen Inc., Germantown, MD).

2.3. Western blot analysis

Cell pellets were lysed on ice in RIPA buffer (Pierce, Rockford, IL), electrophoresed and transferred to a nitrocellulose membrane. Membranes were blocked, incubated with primary antibody, followed by a horseradish peroxidase (HRP) coupled secondary antibody and developed with Visualizer Western Blot Detection Kit (Millipore, Billerica, MA). The following antibodies were utilised: human anti-PLK1 rabbit (1:1000) (Cell Signaling, Danvers, MA); mouse anti-actin (1:2500) (Millipore); goat anti-rabbit-HRP (1:10000) and goat anti-mouse-HRP (1:10000) (Santa Cruz Biotechnology, Santa Cruz, CA).

2.4. Clonogenic assay

Cells were seeded into six-well tissue culture plates and allowed to attach for 6 h. GSK461364A or DMSO control was added to the culture media for 2 h. For combination treatment, GSK461364A or DMSO control was added to the culture media for 2 h followed by treatment with ionising radiation. A detailed protocol is given in the Supplementary methods. For clonogenic assays with RNAi, cells were transfected, trypsinised 48 h post-transfection and plated as described. The seeding density ranged from 200 cells to 6000 cells for GSK461364A treatment and 200 cells to 3200 cells involving RNAi clonogenics.