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Identification of repaglinide as a therapeutic drug for glioblastoma multiforme

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ABSTRACT

Glioblastoma multiforme (GBM) is a highly aggressive brain tumor with a median survival time of only 14 months after treatment. It is urgent to find new therapeutic drugs that increase survival time of GBM patients. To achieve this goal, we screened differentially expressed genes between long-term and short-term survived GBM patients from Gene Expression Omnibus database and found gene expression signature for the long-term survived GBM patients. The signaling networks of all those differentially expressed genes converged to protein binding, extracellular matrix and tissue development as revealed in BiNGO and Cytoscape. Drug repositioning in Connectivity Map by using the gene expression signature identified repaglinide, a first-line drug for diabetes mellitus, as the most promising novel drug for GBM. In vitro experiments demonstrated that repaglinide significantly inhibited the proliferation and migration of human GBM cells. In vivo experiments demonstrated that repaglinide prominently prolonged the median survival time of mice bearing orthotopic glioma. Mechanistically, repaglinide significantly reduced Bcl-2, Beclin-1 and PD-L1 expression in glioma tissues, indicating that repaglinide may exert its anti-cancer effects via apoptotic, autophagic and immune checkpoint signaling. Taken together, repaglinide is likely to be an effective drug to prolong life span of GBM patients.

1. Introduction

Glioblastoma multiforme (GBM) is the most common and lethal type of primary brain tumors in humans with a median survival time of no more than 14 months after standard therapy including surgery, radiotherapy and chemotherapy with temozolomide [1]. Although the genetic/epigenetic alterations in GBM have been extensively studied, identification of effective therapeutic targets proves difficult [2–4]. The outcomes of biological therapeutic approaches such as immunotherapy and anti-angiogenic therapy are also disappointing [5–7]. Therefore, there is an urgent need to develop new therapeutic drugs in order to improve the life span of GBM patients.

With the advance of sequencing techniques, gene expression data are rapidly accumulating. The tremendous genomic resources and bioinformatics approaches have made it more economical and efficient to find drugs [8]. Connectivity Map (CMap) is an effective computational drug repositioning tool, assembling gene expression signature (a group of significantly differentially expressed genes) of specific pathological conditions/diseases with a reference collection of gene expression profiles from cells that have been treated with different drugs [9–11]. Previous studies have reported the successful applications of CMap in identifying therapeutic drugs [8–11].

Abbreviations: CMap, connectivity map; DE genes, differentially expressed genes; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; GBM, glioblastoma multiforme; GEO, Gene Expression Omnibus; IHC, immunohistochemistry; IOD, integral optical density; IC_{50}, half maximal inhibitory concentration; i.p., intraperitoneal injection; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PD-L1, programmed cell death ligand 1; RMA, multi-array analysis.

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Repaglinide, an oral insulin secretagogue for patients with type 2 diabetes mellitus, reduces postprandial glucose excursions by promoting early-phase insulin release from beta-islet cells in pancreas. A nested case-control study shows that repaglinide does not influence the risk of cancer [12] but may have cytotoxic effects against hepatic, breast and cervical carcinoma cells [13], which might be associated with repaglinide-induced oxidative stress, inflammatory mediators and apoptotic genes [14].

In present study, we found gene expression signature in long-term survived patients and identified repaglinide via CMap platform. The anti-cancer effects of repaglinide were verified in vitro and in vivo. Further, the therapeutic mechanisms of repaglinide in GBM were explored.

2. Materials and methods

2.1. Identification of differentially expressed genes from the GEO database

In the present study, the gene expression profiles of GBM patients were obtained from the Gene Expression Omnibus (GEO) database [15] and divided into the long-term (>506 d) and short-term (7–206 d) survived groups. The Expression Console and Transcriptome Analysis Console v3.0 softwares (Affymetrix Company) were used to identify differentially expressed genes (DE genes) between the different survival time groups. The data were filtered with the Fold Change of the gene median expression value ≥ 2 and the ANOVA p-value (Condition pair) < 0.05. The co-expression network construction of all DE genes were built by BiNGO (a biological networks gene ontology tool) [16] and visualized in Cytoscape [17].

2.2. Drug repositioning via CMap

The top 20 up- and down-regulated DE genes were designated as the gene expression signature of long-term survived GBM patients. These signature genes were used to query similar gene expression profiles of drug-treated cells in CMap and then a ranked list of compounds/drugs with connectivity scores and specificity were fed back [9–11]. Repaglinide, a clinical used drug for diabetes but not cancers, was ranked top 6 on the repositioning drug list (Table 1).

2.3. Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to measure cell viability as previously reported [18]. Human GBM cells (LN229) were seeded in 96-well cultured plates (3000 cells/well). Twenty-four hours after seeding, drugs or vehicle at various concentrations were added to the cultures in six parallel wells. The cultures were then incubated for 24, 48 or 72 h before MTT assay (20 mg/ml MTT). Cell viability was determined by light absorption at 490 nm.

2.4. Scratch-wound culture model and cell migration assay

Confluent LN229 cell cultures in 35-mm dishes were used for scratch-wound assay as previously reported [19]. Three parallel scratch lines were drawn with yellow pipette tips and boundaries of the wounds were marked. After DMEM washing, the cultures were incubated with fresh DMEM supplemented with 1% FBS and various concentrations of repaglinide (5, 10, 25 or 50 μM) or vehicle (i.e., 0 μM) for 24 h. The width of wounds was measured with the Image-Pro Plus software and the healing/migration rate was calculated. Statistical results were obtained from triplicate samples in three independent experiments.

2.5. Orthotopic mouse models of GBM

All animal handling and experiments were performed in accordance with NIH guidelines and reviewed by the Ethics Committees of Huazhong University of Science and Technology (HUST). The mice were group housed in the Animal Core Facility of Tongji Medical College with a 12-h light/dark cycle with ad libitum access to food and water. Briefly, adult mice (25–30 g) were anesthetized with chloral hydrate (350 mg/kg) and a burr hole was drilled in the skull 0.5 mm posterior to the bregma and 2.3 mm lateral to the midline. A 10-μl Hamilton syringe (26 gauge, Reno, NV) containing 5×10^5 GL261 cells (mouse GBM cell line) in 2 μl of DMEM was advanced to a depth of 2.6 mm from the skull surface and then withdrawal 0.3 mm. Cell suspension was delivered at the rate of 1 μl/min. After cell implantation, the needle was left in place for 5 min before withdrawal. Repaglinide (1.04 mg/kg) or vehicle was administrated daily via intraperitoneal injection (i.p.) after GBM cell implantation. Animals were observed daily until death or sacrifice.

2.6. Immunohistochemistry (IHC)

IHC was performed as previously reported [20]. Animals were sacrificed 20 days after GBM cell implantation and mouse brains embedded in paraffin were cut into 3 μm-thick slices for IHC analysis. Briefly, the slices were deparaffinized in xylene and antigen-retrieved by microwave processing. After 1 h of blocking

Table 1
The connectivity scores and ranks of the top 15 therapeutic small molecules.

Rank	Small molecule	Connectivity score	Specificity	Cell	Dose
2	Resveratrol	−0.676	0.0278	MCF7	50 μM
4	Valproic acid	−0.261	0.1313	MCF7	50 μM
8	Methotrexate	−0.621	0.0137	MCF7	9 μM
10	PNU-0230031	−0.609	0.0104	MCF7	10 μM
16	Procaine	−0.703	0.0355	MCF7	15 μM
18	Repaglinide	−0.768	0.0098	MCF7	9 μM
19	0173570–0000	−0.641	0.0876	MCF7	10 μM
20	Levonorgestrel	−0.638	0.0656	MCF7	13 μM
21	Pridinol	−0.748	0.0054	MCF7	10 μM
22	LY-294002	−0.209	0.6258	MCF7	10 μM
24	Monobenzone	−0.739	0.0203	MCF7	20 μM
26	Natamycin	−0.735	0.0508	MCF7	6 μM
27	Tetrandrine	−0.726	0.037	MCF7	6 μM
29	4,5-dianilinophthalimide	−0.921	0	MCF7	10 μM
30	Gossypol	−0.606	0.0909	MCF7	8 μM

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