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Inhibition of the insulin-like growth factor 1 receptor by CHM-1 blocks proliferation of glioblastoma multiforme cells

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ABSTRACT

The insulin-like growth factor-1 receptor (IGF-1R) plays a pivotal role in transformation, growth, and survival of glioblastoma multiforme (GBM) cells, and has emerged as a general and promising target for cancer treatment. In this study, we examined the anti-tumor effects of CHM-1, a synthetic 6,7-methylenedioxy substituted 2-phenyl-4-quinolone derivative, on GBM cells *in vitro* and *in vivo*. CHM-1 selectively blocked IGF-1R auto-phosphorylation, with an ability to distinguish between IGF-1R and related tyrosine kinase receptors, such as insulin receptor (IR), epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and fibroblast growth factor receptor (FGFR). Further investigation revealed that, the phosphorylation of ERK1/2 as well as Akt was inhibited in CHM-1 treated GBM8401 cells possibly through the selective blockage of IGF-1R auto-phosphorylation. Our study also showed that p.o. treatment with the hydrophilic dihydrogen phosphate CHM-1P reduced the tumor volumes of the GBM8401 derived tumors in mouse brain and also prolonged the survival rate. The results provided potential opportunities for effective chemotherapy for GBM.

1. Introduction

Glioblastoma multiforme (GBM) is the most aggressive and highly vascularized primary brain tumor in adults [1]. With its highly aggressive nature, GBM is characterized by marked angiogenesis and extensive tumor cell invasion into the normal brain parenchyma [2,3]. Despite advances in surgery, radiotherapy and chemotherapy, the overall prognosis for GBM patients remains dismal. Therefore, new therapeutic studies on the treatment of GBM are still an energetic topic.

The type 1 insulin-like growth factor receptor (IGF-1R) is overexpressed in some human cancers [4–6], including GBM. IGF-1R has been reported to involve in metabolism and survival of GBM cells [7]. Upon the ligands (IGF-1, IGF-2) binding, tyrosine phosphorylation occurs in the kinase domain of IGF-1R and subsequently leads to multisite auto-phosphorylation [8,9]. IGF-1R auto-phosphorylation activates the downstream signal pathways, such as phosphatidylinositol 3′ kinase (PI3K)/Akt and Ras/Raf/mitogen-activated protein kinase (MAPK) which can lead to cell proliferation and anti-apoptotic signaling [10]. According to current understanding, the IGF-1R is not unique in driving tumor cell proliferation, it is required for cellular transformation by most

Abbreviations: EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; GBM, glioblastoma multiforme; IGF-1R, insulin-like growth factor-1 receptor; IR, insulin receptor; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3′ kinase; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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oncogenes and mediates the combination of proliferation and survival signaling required for anchorage-independent growth [11].

Recently, the IGF axis has been recognized as a credible drug target for anticancer therapy and could potentially be hindered at different levels, including reduction of ligand levels or bioactivity, inhibition of receptor function using receptor-specific antibodies, and small-molecular tyrosine kinase inhibitors [12,13]. Ligand targeting approaches involve several measures that attempt to lower ligand concentration or use ligand-specific antibodies [14]. As for receptor-specific antibodies, several of them are being evaluated in clinical trails. The IGF-1R blocking antibodies have revealed favorable toxicity and available clinical results such as Pfizer antibody CP-751871 combining with chemotherapy in squamous cancers, which raises the response rate to chemotherapy alone of 41–72% with the antibody [15–17].

On the basis of tyrosine kinase inhibitor strategy, the design is complicated by the fact that the kinase domain of IGF-1R shares 85% similarity with that of insulin receptor (IR), and the ATP binding cleft is 100% conserved [18]. Lacking the targeting selectivity could lead to the possibility of serious metabolic toxicity due to the blockage of IR signaling. Fortunately, several promising agents which employ as inhibitors of substrate phosphorylation show greater potential for specific IGF-1R inhibition, for instance, cyclolignan picropodophyllin (PPP), which inhibits tyrosine phosphorylation of Y1136 in the activation loop of the IGF-1R kinase domain, and does not affect the IR [19].

Quinolone derivatives have been discovered to have anti-cancer activities by the inhibition of tubulin polymerization and antimitotic performance [20–22]. Until now, some 2-phenylpyrroloquinolin-4-ones have been synthesized from the 2-phenyl-4-quinolones and shown to inhibit the growth of hepatocellular carcinoma both *in vitro* and *in vivo* [23]. In particular, CHM-1 significantly inhibited tubulin polymerization and showed cytotoxic effect on osterogenic sarcoma, colorectal adenocarcinoma, hepatocellular carcinoma, and ovarian cancer cell lines [24–27]. Although the extensive studies of CHM-1 have been carried out in various tumor cells, less information is known in GBM cells. In this study, we demonstrated the anti-cancer activity and cytotoxic effects of CHM-1 on human GBM cells and its underlying novel molecular mechanism. The results revealed that CHM-1 is a highly potent inhibitor for the growth of GBM cells.

2. Materials and methods

2.1. Antibodies and reagents

Quinolone derivatives, CHM-1, and CHM-1P were synthesized in our laboratory. Antibodies and reagents were purchased from commercial sources: antibodies against pY1135/1136-IGF-1R, IGF-1R β subunit, IR β subunit, pS2448-mTOR, pS473-Akt, Akt, pY202/204-ERK1/2, ERK1/2, pT389-p70S6K, and p-4E-BP1 were purchased from Cell Signaling Technology (Beverly, MA, USA); antibody against epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), phosphotyrosine (PY99), anti-mouse and anti-rabbit antibodies conjugated to horseradish peroxidase were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); antibody against cyclin D1 was purchased from Abcam (Cambridge, UK); anti-β-actin antibody was from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Cell lines and cell cultures

The human DBTRG-05MG cells were obtained from American Type Culture Collection (Rockville, MD, USA). The human GBM8401, GBM8901, and G5T/VGH cells were obtained from Bioresources Collection and Research Center (Hsinchu, Taiwan). These cells were cultured in DMEM/F-12 supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin, and were grown at 37 °C in a humidified atmosphere of 5% CO_2.

2.3. Western blot analysis and immunoprecipitation

Cells were treated with various agents as indicated in the figure legends. After treatment, Western blotting was performed as described previously [28]. For the evaluation of receptor tyrosine phosphorylation, 0.5 mg of cell lysate protein was first immunoprecipitated with the antibodies. Subsequently, immunoprecipitates were resolved by a 7.5% SDS–PAGE and probed with the PY99 anti-phospho-tyrosine MAb. The intensity of the bands was scanned and quantified with National Institutes of Health Image software.

2.4. Cell proliferation assays

The effect of cell proliferation was examined by MTT method as described previously [29]. In brief, cells were treated with different concentrations of drugs. After incubation for various times, MTT solution (5 mg/mL, Sigma Chemical Co., St Louis, MO, USA) was added to each well and incubated for 2 h at 37 °C. The MTT-formazan crystals formed by metabolically viable cells were dissolved in DMSO. Finally, the absorbance was monitored by a microplate reader at a wavelength of 570 nm.

2.5. Molecular flexible docking

Molecular flexible docking was performed by Dock 5.1 [30]. The Kollman partial charges were applied to protein models for force field calculation. Energy-optimized 3D coordinates and partial charges of small molecules were calculated by Marvin 5.2.2, Balloon 0.6 and OpenBabel 2.2.3 [31,32]. There were 1000 orientations searched and 200 conformers generated per cycle identified in Dock program. The docked conformers were then re-scored by HotLig to predicted protein–ligand binding modes. Protein structural superimposition was calculated and represented by Chimera 1.4.1 [33].

2.6. In vivo S.C. xenografts

The animals used had access to food and water ad libitum. Experimental procedures using animals were approved by the Institutional Animal Care and Use Committees of The China Medical University.

Female BALB/c nu/nu mice (18–20 g; 6–8 weeks of age), were purchased from National Animal Center (Taipei, Taiwan) and maintained in pressurized ventilated cage according to institutional regulations. GBM8401 or GBM8901 cells in log phase growth (2×10^6 cells) were inoculated subcutaneously into the right flank of the mice. When tumors reached an approximate volume of 100 mm^3, mice were selected and distributed for drug studies. CHM-1P was diluted with sterile saline before injection. Mice were weighed and tumors were measured using calipers every 3 days. Tumor volumes were determined by measuring the length (l) and the width (w) and the volumes were calculated as $V = lw^2/2$. On the final day of the treatment, mice were sacrificed, tumors were excised, weighted and sectioned and the tumor sections were embedded in OCT (optimum cutting temperature) compound and frozen at −70 °C.

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