Identification of cephalomannine as a drug candidate for glioblastoma via high-throughput drug screening

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SUMMARY

Glioblastoma (GBM) is the most common malignant primary tumor of the central nervous system in adults. Despite advances in GBM treatment, the prognosis of patients with GBM remains poor and novel drugs are urgently required. In this study, we aimed to identify novel drugs for GBM treatment by using a drug screening approach. To this end, we performed high-throughput screening with 118 drugs, including Food and Drug Administration (FDA)-approved anticancer drugs. We found high inhibition rates (more than 90%) for doxorubicin, bortezomib, and cephalomannine in 6 GBM cell lines. Furthermore, we determined the half-maximal inhibitory concentration (IC50) of cephalomannine and found that the drug has a high potential for anti-GBM activity. Moreover, we noted that cephalomannine inhibited cell proliferation by inducing autophagy. Thus, our results indicate that cephalomannine may be an effective drug candidate for GBM treatment.

Key words: glioblastoma, high-throughput screen, cephalomannine

INTRODUCTION

Glioblastoma (GBM) is the most common and invasive malignant primary brain tumor in adults.1, 2) Its prognosis remains poor, even with the current standard of care, i.e., a combination of surgery, radiotherapy, and chemotherapy.3, 4) The disease course of GBM is typically rapid, and it has a 5-year survival rate of only 10% and a median survival time of 15 months following intensive treatment.5–8) Therefore, there remains a strong need for novel therapeutic strategies to treat GBM.

The discovery of new compounds for medical conditions is generally time-consuming and extremely expensive. Compared to the traditional methods of developing new drugs, the drug repurposing strategy, which helps identify new indications for existing drugs, could not only be time-saving but also cost-effective. High-throughput screening (HTS) is an effective strategy for drug discovery. Several reports regarding the characteristics, methodology, and evaluation of HTS have been previously published.9, 10) The innovation and optimization of HTS methods related to translational sciences play very important roles in drug discovery. Because cancer cells can be characterized by their ability to proliferate, an available approach to HTS for anticancer activity is to screen the compounds that inhibit the proliferation of cancer cells in culture. This method allows the identification and validation of compounds that target factors involved in the development of numerous types of cancer. Recent examples of the successful use of cell-based screens were with various types of cancer cells. Iljin et al. identified disulfiram as an inhibitor of prostate cancer through HTS.11) Guzmán et al. reported that the marine-derived compound batzelline was selectively cytotoxic to pancreatic cells lines over the normal kidney epithelial cell line.12) A previous study has also used this approach to identify compounds with the ability to treat GBM.13) However, none of these compounds is used in clinical practice. Therefore, novel agents are required to improve the therapeutic outcome for patients of GBM. In this study, we aimed to identify novel agents for GBM treatment by using HTS.

MATERIALS AND METHODS

Cells and culture

In this study, six human glioblastoma cell lines were used.

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T98G, KS-1, YH-13, and SF126 cell lines were purchased from the Japanese Collection of Research Bioresources (JCRB), and A172 and AM-38 cell lines were purchased from the RIKEN cell bank. The KS-1, YH-13, SF126, and AM-38 cell lines were maintained in Eagle’s minimum essential medium (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), and the T98G and A172 cell lines were maintained in Roswell Park Memorial Institute (RPMI) medium (Sigma-Aldrich). The cells were cultured at 37°C in a humidified atmosphere with 5% CO₂. Cells from exponentially growing cultures were used in all experiments.

Drug screening for cell growth inhibition

Cells were seeded duplicated in a 384-well culture plate (4,000 cells/well) and grown overnight at 37°C under an atmosphere with 5% CO₂. One hundred and eighteen low-molecular-weight chemical compounds, which included Food and Drug Administration (FDA)-approved drugs (Selleck Chemicals, Houston, TX), were added using a Bravo Automated Liquid Handling Platform (Agilent Technologies, Santa Clara, CA). After treatment for 72 h, living cells were analyzed using a cell counting kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan). The experiment was performed in duplicate. The anticancer drugs used in this study are listed in Supplementary Table 1. Subsequently, to determine the IC₅₀ value of cephalomannine, the tumor cells were treated with various concentrations of cephalomannine for 72 h. Cell proliferation was then measured using CCK-8. IC₅₀ values were determined using four-parameter curve fitting with GraphPad Prism (version 7; GraphPad Software, San Diego, CA, USA).

Western blotting

Protein samples (10 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on a 12.5% polyacrylamide gradient gel (Atto, Tokyo, Japan) and subsequently blotted onto a nitrocellulose membrane (Bio-Rad). The membranes were blocked with 5% skim milk in 20 mM Tris-buffered saline (TBST, 0.1% Tween 20) for 1 h at room temperature and then incubated overnight at 4°C with the mouse anti-LC3 primary antibody (1:1,000; purchased from MBL International, Woburn, MA, USA, M152-3). After incubation, the membranes were rinsed five times with TBST buffer and incubated for 1 h with the secondary antibody, anti-mouse IgG antibody (1:2,000; Sigma, St Louis, MO, USA). To normalize the variance between the loading samples, reversible Ponceau S (Sigma) staining was performed for the membranes. Immunocomplexes were detected using the ECL Prime Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK) by using Amersham Imager 600 (GE Healthcare).

Statistical analysis

Results were expressed as the mean±standard error of means (SEM) of at least three independent experiments. Differences between the control and variable conditions were determined by two-tailed Student’s t-tests and one-way analysis of variance, and results were considered to be significant at P<0.05.

RESULTS

Drug screening in GBM cell lines

Using six GBM cell lines, namely KS-1, YH-13, SF126, AM-38, T98G, and A172, we screened 118 drugs, including FDA-approved drugs, at a fixed concentration (10 μM, Fig. 1). When the threshold was set at 50% cell viability, three drugs, bortezomib, cephalomannine, and doxorubicin, showed remarkable growth-suppressive effects on the six cell lines, including (Table 1). The corresponding average inhibition rates were 97.45%, 90.61%, and 83.14%.

Table 1. Cell proliferation inhibition rate (%) of bortezomib, cephalomannine, and doxorubicin in six GBM cell lines.

<table>
<thead>
<tr>
<th>Drug</th>
<th>SF126</th>
<th>AM38</th>
<th>KS-1</th>
<th>YH13</th>
<th>A172</th>
<th>T98G</th>
<th>Average inhibition rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bortezomib</td>
<td>98.17</td>
<td>94.18</td>
<td>98.03</td>
<td>97.25</td>
<td>97.71</td>
<td>99.39</td>
<td>97.45</td>
</tr>
<tr>
<td>Cephalomannine</td>
<td>86.07</td>
<td>77.74</td>
<td>91.60</td>
<td>96.75</td>
<td>96.31</td>
<td>95.20</td>
<td>90.61</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>95.21</td>
<td>92.77</td>
<td>97.50</td>
<td>90.50</td>
<td>65.91</td>
<td>56.97</td>
<td>83.14</td>
</tr>
</tbody>
</table>
Sensitivity of the six GBM cell lines to cephalomannine treatment

To verify the sensitivity of GBM cells to cephalomannine, the GBM cell lines were treated with various concentrations of cephalomannine for 72 h, and cell viabilities were determined using the CCK8 assay. The percentage of surviving cells decreased in a dose-dependent manner in all the cell lines (Fig. 2A). The 72-h IC₅₀ values of cephalomannine for the A172, AM-38, KS-1, SF126, T98G, and YH-13 cell lines were 35.1, 27.1, 12.4, 25.9, 25.2, and 10.9 nM respectively (Fig. 2B).

Induction of autophagy of GBM cells by cephalomannine

A previous report showed paclitaxel, an analog of cephalomannine, inhibited growth and induced autophagy in gastric cancer cells. Therefore, we examined if cephalomannine induced autophagy of GBM cells. The T98G cells were treated with various concentrations of cephalomannine for 72 h. Autophagy was detected by measuring LC3-I and LC3-II by western blotting. The results showed that the ratio of LC3-II to LC3-I in the 25 or 50 nM cephalomannine group was 172.1% or 153.1% higher than that in the control sample. There was no significant difference between the 5 or 10 nM cephalomannine group and the control sample (Fig. 3A and 3B). Western blots revealed that cephalomannine treatment led to an increase in LC3-II in T98G cells. These results clearly showed that cephalomannine induces autophagy of GBM cells.

**DISCUSSION**

Using HTS, a powerful tool in drug discovery, we demonstrated that cephalomannine is a candidate anti-GBM drug. Our in vitro assay provides clear evidence for the use of cephalomannine in GBM treatment. Our results also suggest that HTS is an effective approach for rapidly identifying novel potential applications of existing drugs.

Cephalomannine is a natural congener of paclitaxel and was isolated in the 1970s from *Taxus wallichiana*, which was erroneously named as *Cephalotaxus mannii* at the time of its discovery. It differs from paclitaxel only in the amide portion; the substituent at the C3 position is tigloyl amide instead of benzamide. Due to structural similarities, cephalomannine shows antitumor activity comparable to that of paclitaxel toward several different cell lines. It is especially effective against leukemia.

**Fig. 2. Cephalomannine suppresses the proliferation of GBM cells.**

Six GBM cell lines were incubated with cephalomannine at various concentrations for 72 h. The relative number of remaining cells was determined using the CCK-8 assay.

**Fig. 3. Effect of cephalomannine on autophagy.**

Western blot analysis of LC3 in T98 cells. The cells were treated with cephalomannine for 24 h. Whole-cell lysates were separated by SDS-PAGE and immunoblotted with the indicated antibodies. Results are representative of three independent experiments.
useful for the development of a more effective therapy.

This study has several limitations. First, since the principal mechanism of action of cephalomannine is the disruption of microtubule function\(^{23}\), it is supposed to be functionally associated with the microtubule. Further work needs to be done to understand its inhibitory mechanisms in GBM. Second, we did not investigate the anti-GBM effect of cephalomannine in vivo. Further investigation of anti-GBM activity of cephalomannine using an animal model is required to assess its in vivo efficacy and safety, which would provide a more detailed understanding of the therapeutic effect of cephalomannine in GBM.

In summary, we identified drug candidates for GBM treatment using an HTS approach and found that cephalomannine can reduce the viability of GBM cells and induce autophagy. Our results highlight the possibility of using cephalomannine for GBM treatment.

**ABBREVIATIONS**

GBM, glioblastoma; JCRB, Japanese Collection of Research Bioresources; FDA, Food and Drug Administration; HTS, High-throughput screening

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**CONFLICT OF INTEREST**

The authors declare no competing financial interests.

**REFERENCES**


