Identification of cantharidin as a drug candidate for glioblastoma by using a Connectivity Map–based approach

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SUMMARY

Glioblastoma (GBM) is the most common brain tumor in adults. Although the surgical and chemoradiotherapy approaches for treatment have improved, the prognosis of patients with GBM is still poor and novel drugs are urgently required. Therefore, we investigated small molecular inhibitors to target GBM on the basis of gene expression data by using a Connectivity Map (CMAP)–based approach. Using meta-analysis performed with publically available gene expression data, we identified the gene expression signature of GBM. The CMAP analysis identified 15 candidate drugs for GBM treatment. We confirmed the anticancer cell proliferation activity of cantharidin as one of the top 15 drugs with high negative enrichment scores in CMAP analysis by using GBM cell lines. Our results indicate the potential utility of CMAP to discover the potent drugs in the GBM treatment. This approach can be applied to other malignancies than GBM.

Key words: glioblastoma, meta-analysis, Connectivity Map, cantharidin

INTRODUCTION

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults, characterized by rapid and infiltrating growth. The current multimodal standard of care using surgery, radiation, and chemotherapy has limited effects on the patients with GBM, especially those at the advanced stage. Although novel treatments for GBM have been challenged from basic research to clinical trials, patients with GBM with poor prognosis of a progression-free survival of 7–8 months, a median survival of 14–16 months and 5-year overall survival of 9.8%

Connectivity Map (CMAP) is a helpful tool for elucidating disease-gene-drug connections by utilizing microarray technology. CMAP includes gene expression signatures of various cultured cancer cell lines treated with a library of small molecule compounds. The CMAP platform links drugs, genes, and diseases by measuring similarity or dissimilarity using gene expression profiling. Using a pattern-matching algorithm, the program can identify drugs predicted to revert the oncogenic gene signature of a given cancer to a nonmalignant or drug-sensitive gene expression profile. The drugs examined in the CMAP are often used in clinical treatment or are well-established drugs. In addition to focusing on a single drug target, the use of a batch of genes to query the CMAP may not only allow multiple targets to be considered simultaneously but may also help identify potential novel drugs. Previous studies have successfully used this approach to identify compounds with the ability to modulate various biological pathways or diseases. These previous reports may suggest that we can identify potential drugs and proceed to the clinical trial stage.

In this study, using in silico drug screening via CMAP, which was followed by validation, we found that cantharidin can reduce the viability of GBM cells and induce apoptosis.

MATERIALS AND METHODS

Cells and culture

This study used four human glioblastoma cell lines, that is, T98G, KS-1, YH-13, and SF126, which were purchased from the Japanese Collection of Research Bioresources (JCRB). The KS-1, YH-13, and SF126 cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM;...
Sigmawith 10% FBS. The T98G cell line was maintained in Roswell Park Memorial Institute (RPMI) medium (Sigma). The cells were cultured at 37°C in a humidified atmosphere with 5% CO₂. Cells from exponentially growing cultures were used in all experiments.

**Cell proliferation assay**

Tumor cells plated on 96 well-plates (10,000 cells/well) were incubated in the presence of the following (used at 10 μM concentration) for 72 h: cantharidin from Abcam (Cambridge, UK); trioxysalen from Sigma; DL-thiorphan from Sigma; dexverapamil from Merck (Fair Lawn, Germany); 1,4-chrysenequinone from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan); and apigenin, menadione, 8-azaguanine, luteolin, piperlongumine, roxithromycin, ronidazole, ginkgolide A, fisetin, and tyloxoapil from Selleck Chemicals (Houston, TX, USA). Then, cell proliferation was measured using Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer’s recommendations. Subsequently, to evaluate the IC50 value of cantharidin, the tumor cells were treated with various concentrations of cantharidin for 72 h. Cell proliferation was then measured using Cell Counting Kit-8. IC50 values were calculated using four-parameter curve fitting with GraphPad Prism (version 7; GraphPad Software, San Diego, CA, USA).

**Microarray data collection**

Gene expression data for glioblastoma and normal brain tissues were obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). GEO data from January 2001 to December 2016 were included in this study. The information provided with the gene expression data in the GEO database and the related publications were used to confirm the characteristics of the samples. The following information was curated for each data set: GEO accession number, publication, DNA microarray platform, number of cases, references, and gene expression data. Studies were eligible for the meta-analysis if they met the following inclusion criteria: (a) use of tissue samples, (b) data available via the platform of the Affymetrix Human Genome U133 Plus 2.0 Array (GPL570), and (c) datasets containing raw files. All the microarray datasets used for meta-analysis are listed in Table 1.

**Analysis of differentially expressed genes (DEGs)**

Gene expression data analysis was performed using the R software and packages from the Bioconductor project. The DNA microarray data were normalized using MAS5.0 with the Bioconductor affy package. The genes differentially expressed (fold change >2, p<0.05) between GBM and normal brain tissue samples were analyzed with the limma package and tested with the modified t-test based on empirical Bayes methods.

**CMAP analysis**

The data for genes differentially up- and downregulated between the two sample sets, as determined from the microarray data, were entered into the online software Connectivity Map (https://www.broadinstitute.org/cmap/). Only drugs that had negative scores and P values of <0.05 were considered for further study. The sum of drug occurrences was used to rank the drugs.

**Western blotting**

Protein samples (10 μg) were separated by sodium do-decyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on a 12.5% polyacrylamide gel (Atto, Tokyo, Japan) and subsequently blotted onto a nitrocellulose membrane (Bio-Rad). The membranes were blocked with 5% skim milk in 20 mM TBST (Tris-buffered saline, 0.1% Tween 20) for 1 h at room temperature and then incubated overnight at 4°C with the following primary antibodies (Abcam): rabbit anti-caspase 3 antibody (1:1000), rabbit anti-caspase 9 antibody (1:1000), or rabbit anti-PARP1 antibody (1:1000). After incubation, the membranes were rinsed five times with TBST buffer and incubated for 1 h with the secondary antibody, that is, anti-rabbit IgG antibody (1:2000; Sigma, St Louis, MO, USA). To normalize the variance of loading samples, reversible Ponceau S (Sigma) staining was performed for membrane staining. Immunocomplexes were detected using the ECL Prime Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK) by using Amersham Imager 600 (GE Healthcare).

**RESULTS**

**Identification of genes differentially expressed between GBM and normal brain samples by meta-analysis**

To identify the gene expression signature of GBM for CMAP analysis, we performed meta-analysis with gene expression data deposited in the GEO database from 2001 to 2016. Using this approach, we eventually compared the data for 431 GBM samples and 101 normal brain samples. A total of 750 genes were found to be differentially expressed between GBM tissues and normal brain tissues; among these, 234 genes were upregulated and 516 genes were downregulated in GBM tissue samples (Fig. 1). The data regarding DEGs have been provided in Supplementary Table 1.

**Identification of new candidate therapeutic compounds by CMAP analysis of gene signatures of GBM**

We hypothesized that if a drug treatment could reverse the gene expression signature of GBM to that present in the normal brain, it might have the potential to inhibit pathways essential in the formation of GBM and could therefore be used to treat GBM. To identify compounds having such an effect, we performed CMAP analysis by searching for negatively correlated gene expression patterns associated with
| GEO       | Title                                                                 | Publication                                                                 | Platform                  | Link GEO                                      |
|----------|----------------------------------------------------------------------|----------------------------------------------------------------------------|---------------------------|----------------------------------------------|
| GSE4290  | Neuronal and glioma-derived stem cell factor induces angiogenesis within the brain. | Sun L, Hui AM, Su Q, Vortmeyer A et al. Cancer Cell 2006 Apr;9(4):287-300. | Affymetrix Human Genome U133 Plus 2.0 Array | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4290 |
| GSE4536  | Tumor stem cells derived from glioblastomas cultured in 3DGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. | Lee J, Kotliarova S, Kotliarov Y, Li A et al. Cancer Cell 2006 May;9(5):391-403. | Affymetrix Human Genome U133 Plus 2.0 Array | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4536 |
| GSE4536  | Stem cell-related “self-renewal” signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. | Murat A, Migliaravaca E, Gorial T, Lamothe WP et al. J Clin Oncol 2008 Jun 20;26(18):3015-24. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7696 |
| GSE4536  | MAP kinase-interacting kinase 1 regulates SMAD2-dependent TGF-β signaling pathway in human glioblastoma. | Grzani M, Morin P Jc, Lino MM, et al. Cancer Res 2011 Mar 15;71(6):2392-402. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4536 |
| GSE19728 | Gene expression profiling in human high-grade astrocytomas. | Liu Z, Yao Z, Li C, Lu Y et al. Comp Funct Genomics 2011;2011:245137. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15824 |
| GSE31545 | Progenitor-like traits contribute to patient survival and prognosis in oligodendroglioma tumors. | Ng FS, Toh TB, Tjog EH, Kooh GR et al. Clin Cancer Res 2012 Aug 1;18(15):4122-25. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE31545 |
| GSE36245 | Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. | Sturm D, Witt H, Hovestadt V, Khourang-Quang DA et al. Cancer Cell 2012 Oct 16;22(4):425-37. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36245 |
| GSE36782 | Glioma-propagating cells as an in vitro screening platform: PLK1 as a case study. | Foong CS, Sandanaraj E, Brooks HB, Campbell RM et al. J Biomed Screen 2012 Oct;17(9):1136-50. PMID: 22927677 | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36782 |
| GSE44843 | Involvement of miRNAs in the Differentiation of Human Glioblastoma Multiforme Stem-Like Cells | Aldar, B., Sagardoy, A., Nogueira, L., Guruceaga, E., Grande, L., Huse, J.T., Aznar, M.A., Diez-Valle, R., Tejada-Solis, S., and Alonso, M.M. (2013). PloS one 8, e77098. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44843 |
| GSE50161 | Characterization of distinct immunophenotypes across pediatric brain tumor types. | Griesinger AM, Birks DK, Donson AM, Amani V et al. J Immunol 2013 Nov 1;191(9):4860-8. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE50161 |
| GSE51062 | Sprouty2 Drives Drug Resistance and Proliferation in Glioblastoma. | Walsh AM, Kapoor GS, Buonato JM, Mathew LK, Sturm D, Witt H, Hovestadt V, Khuong-Quang DA et al. Cancer Cell 2012 Aug 16;22(4):425-37. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51062 |
| GSE53373 | MDSCs mediate angiogenesis and predispose canine mammary tumor cells for metastasis via IL-28/IL-28RA (IFN-α) signaling. | Mucha, J., Majchrzak, K., Taciak, B., Hellmén, E., and Kröl, M. (2014). PloS one 9, e103249. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53373 |
| GSE86574 | Characterization of 2 Novel Ependymoma Cell Lines With Chromosome 1q Gain Derived From Posterior Fossa Tumors of Childhood. | Amani, Y., Donson, A.M., Lummus, S.C., Prince, E.W., Griesinger, A.M., Witt, D.A., Hankinson, T.C., Handler, M.H., Dorris, K., Vibhakar, R., et al. (2017). J Neuropathol Exp Neurol 76, 595-604. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE86574 |
| GSE35493 | Pediatric rhabdoid tumors of kidney and brain show many differences in gene expression but share dysregulation of cell cycle and epigenetic effector genes. | Birks DK, Donson AM, Patel PR, Sutli A et al. Pediatr Blood Cancer 2013 Jul;60(7):1095-102. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35493 |
| GSE35864 | The National NeuroAIDS Tissue Consortium brain gene array; two types of HIV-associated neurocognitive impairment. | Gelman BB, Chen T, Lisinichia JG, Soukup VM et al. PLoS One 2012;7(9):e16178. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35864 |
| GSE50161 | Characterization of distinct immunophenotypes across pediatric brain tumor types. | Griesinger AM, Birks DK, Donson AM, Amani V et al. J Immunol 2013 Nov 1;191(9):4860-8. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE50161 |
| GSE66354 | Interleukin-6/STAT3 Pathway Signaling Drives an Inflammatory Phenotype in Group A Ependymoma. | Griesinger AM, Josephson RJ, Donson AM, Mc Laughly Levy J et al. Cancer Immunol Res 2015 Oct;3(10):1165-74. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66354 |
| GSE68015 | Identification of targets for rational pharmacological therapy in childhood cranioopharyngioma. | Gump JM, Donson AM, Birks DK, Amani VM et al. Acta Neuropathol Commun 2015 May 21;3:30. | Affymetrix Human Genome U133 Plus 2.0 Array | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE68015 |
drug-treated cancer cells. The gene signatures of GBM were used as input query items for comparison with those related to drug treatments in the CMAP database. Several drugs were found to reverse the signatures of GBM to those of the normal brain. The top 15 drugs with high negative enrichment scores have been mentioned in Table 2.

Characterization of potential drugs for GBM via cell viability assays

To examine whether the candidate compounds have antitumor effects on GBM, we tested the top 15 drugs on the four GBM cell lines T98G, SF126, KS-1, and YH13 and determined cell viability with the CCK8 assay. First, we examined the effects of each drug on the proliferation of the four cell lines when used at the concentration of 10 μM. Different inhibitory effects were observed for the 15 drugs (Fig. 2). We found that cantharidin had the highest inhibitory effect on all the cell lines. Second, to verify the sensitivity of GBM cells to cantharidin, the cell lines were treated with various concentrations of cantharidin for 72 h, and cell viability was determined. The percentage of surviving cells decreased in a dose-dependent manner in all the cell lines (Fig. 3). The 72-h half maximal inhibitory concentrations (IC50) of cantharidin in the T98G, SF126, KS-1, and YH13 cells were 0.295, 1.51, 0.673, and 0.428 μM, respectively. These results suggest that cantharidin could be the strongest candidate drug for GBM treatment.

Induction of apoptosis of GBM cells by cantharidin

We examined if cantharidin induced apoptosis of GBM cells, because cantharidin induced apoptosis in various malignancies16–18). Apoptosis was detected by measuring cleaved caspase-3, caspase-9, and PARP1 with western blotting. Caspase-9 is an initiator caspase that, following

| Table 2. Top 15 candidate compounds for GBM, as determined by CMAP analysis (p<0.05). |
|---|---|---|---|
| Rank | CMAP name | Enrichment | P-value |
| 1 | cantharidin | −0.944 | 0 |
| 2 | apigenin | −0.941 | 0.00712 |
| 3 | menadione | −0.896 | 0.03338 |
| 4 | dexverapamil | −0.872 | 0 |
| 5 | 1,4-chrysenequinone | −0.87 | 0.0476 |
| 6 | 8-azaguanine | −0.841 | 0.01251 |
| 7 | luteolin | −0.826 | 0.00209 |
| 8 | piperlongumine | −0.816 | 0.03247 |
| 9 | roxithromycin | −0.801 | 0.0367 |
| 10 | ronidazole | −0.757 | 0.01064 |
| 11 | trioxsalen | −0.72 | 0.02598 |
| 12 | ginkgoide A | −0.688 | 0.02286 |
| 13 | DL-thiorphan | −0.68 | 0.04044 |
| 14 | fisetin | −0.672 | 0 |
| 15 | tyloxapol | −0.647 | 0.02039 |

Fig. 1. Identification of gene expression signatures in GBM by bioinformatics analysis. The heat map shows the genes differentially expressed between GBM (n=431) and normal brain tissue samples (n=101). Red indicates high relative expression and green indicates low relative expression. 2, 0, and −2 are the fold changes in the corresponding spectrum.

Fig. 2. Candidate therapeutic compounds screened in vitro with human GBM cells. T98G, KS-1, YH-13, and SF126 cells were treated with the top 15 compounds with high negative enrichment score identified by CMAP analysis (10 μM) for 72 h.

Fig. 3. Cantharidin suppresses proliferation of GBM cells. Four GBM cell lines were incubated for 72 h with cantharidin used at various concentrations. The relative number of the remaining cells was evaluated using the CCK-8 assay.
that thioridazine can serve as a drug candidate for GBM. They identified the gene expression signatures of GBM by using five public gene expression datasets as the input query\(^\text{23}\). The candidate compounds identified by Cheng et al. differ from anticancer drugs we identified, probably because the input query was different. Cheng et al. examined 197 GBM and 33 normal brain tissue from 4 studies, and we examined the results for 431 GBM and 101 normal brain tissue samples from 18 studies by exploring the GEO database.

Cantharidin is an active constituent of mylabris, a traditional Chinese medicine\(^\text{24}\). It is a potent and selective inhibitor of protein phosphatase 2A (PP2A), which plays an important role in the control of the cell cycle, apoptosis, and cell-fate determination\(^\text{25–27}\). Therapeutic relevance of the protein phosphatase 2A was reported in different types of cancers including GBM, and the possible therapeutic utility of PP2A inhibitor was suggested in the previous reports\(^\text{28, 29}\). Thus our CMAP analysis and following in vitro experiments confirmed the potential therapeutic utility of cantharidin in GBM and the utility of CMAP analysis in the drug discovery.

In this study, we found that cantharidin induces apoptosis of GBM cells. Several previous studies have reported the mechanism of cantharidin-induced apoptosis in different types of cancers. Sagawa et al. reported that cantharidin induces apoptosis of human multiple myeloma cells via inhibition of the JAK/STAT pathway\(^\text{30}\). Hsia found that cantharidin induces apoptosis of H460 human lung cancer cells through mitochondrion-dependent pathways\(^\text{16}\). Wang et al. showed that cantharidin impaired pancreatic cancer cells by repressing the β-catenin pathway\(^\text{31}\). Further studies including the mechanisms of inhibitory effects of cantharidin will be important to apply the in vitro data to the clinical applications.

In summary, we identified potential drug candidates for GBM by using a CMAP–based approach. As a proof of concept, we confirmed that cantharidin reduced the viability of GBM cells and induced apoptosis. The other identified drugs are worth investigating, and this approach can be applied to other malignancies than GBM.

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**CONFLICT OF INTERESTS**

The authors declare no competing financial interests.

**ABBREVIATIONS**

GBM, glioblastoma; CMAP, Connectivity Map; JCRB, Japanese Collection of Research Bioresources; GEO, Gene activation, cleaves procaspase-3 and procaspase-7, with ensuing cleavage of several cellular targets, including PARP1. PARP1 serves as a substrate for both caspase-3 and caspase-7; cleaved PARP1 is a hallmark of caspase-dependent apoptosis\(^\text{19}\). Western blots revealed that cantharidin treatment led to a dose-dependent increase in cleaved caspase-3, caspase-9, and PARP1 in T98G cells (Fig. 4). These results clearly showed that cantharidin induces apoptosis of GBM cells.

**DISCUSSION**

In the current study, using CMAP analysis, a powerful bioinformatics tool in drug discovery, we showed that cantharidin is a candidate anti-GBM drug. Our in vitro function assay clearly provides evidence for the usage of cantharidin for GBM treatment. Our results also suggest that expression-based in silico screening with CMAP is an effective approach for rapidly identifying novel potential applications for existing drugs.

The discovery of new compounds for medical conditions is generally time-consuming and extremely expensive; therefore, new strategies for drug discovery are urgently required. Compared with the traditional methods for 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. CMAP is an important method in drug repurposing as it helps establish connections among genes, drugs, and diseases. Recently, some studies successfully identified and validated potential therapeutic compounds for several different tumors by CMAP analysis\(^\text{20–22}\). Using this method, Cheng et al. found...
REFERENCES

1) Hutchinson L. Genetics: New paediatric glioblastoma treatment option. Nat Rev Clin Oncol. 2017;14:4.
2) Weller M, Wick W, Aldape K, et al. Glioma. Nature reviews Disease primers. 2015;1:15017.
3) Noroxe DS, Poulsen HS, Lassen U. Hallmarks of glioblastoma: a systematic review. ESMO open. 2016;1:e000144.
4) Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009;10:459–466.
5) Michaelsen SR, Christensen IJ, Grunnet K, et al. Hallmarks of glioblastoma: a systematic review. ESMO open. 2016;1:e000144.
6) Ju SY, Huang CY, Huang WC, Su Y. Identification of thiostrepton as a novel therapeutic agent that targets human colon cancer stem cells. Cell Death & Disease. 2015;6:e1801.
7) Reimers M, Carey VJ. Bioconductor: an open source framework for bioinformatics and computational biology. Methods Enzymol. 2006;411:119–134.
8) Gautier L, Cope L, Bolstad BM, Irizarry RA. Affy-analyses of Affymetrix GeneChip data at the probe level. Bioinformatics. 2004;20:307–315.
9) Dudoit S, Gilbert HI, van der Laan MJ. Resampling-based empirical Bayes multiple testing procedures for controlling generalized tail probability and expected value error rates: focus on the false discovery rate and simulation study. Biostat J. 2008;50:716–744.
10) Reimers M, Carey VJ. Bioconductor: an open source framework for bioinformatics and computational biology. Methods Enzymol. 2006;411:119–134.
11) Hsia TC, Yu CC, Hsu SC, et al. Cantharidin induces apoptosis of H460 human lung cancer cells through mitochondria-dependent pathways. Int J Oncol. 2014;45:245–254.
12) Malcomson B, Wilson H, Veglia E, et al. The Connectivity map links the regulatory protein-1-mediated inhibition of hypoxia-inducible factor-2a translation to the anti-inflammatory 15-deoxy-delta12,14-prostaglandin J2. Cancer Res. 2010;70:3071–3079.
13) Cheng HW, Liang YH, Kuo YL, et al. Identification of thioridazine, an antipsychotic drug, as an antiglioblastoma and anticancer stem cell agent using public gene expression data. Cell Death Dis. 2015;6:e1753.
14) Wang G, Dong J, Cai H, Wang W. Cantharidin as an antitumor agent: a retrospective review. Curr Med Chem. 2013;20:159–166.
15) Wang J, Li X, Chen Z, et al. Cantharidin, a potent and selective PP2A inhibitor, induces an oxidative stress-independent growth inhibition of pancreatic cancer cells through G2/M cell-cycle arrest and apoptosis. Cancer Sci. 2010;101:1226–1233.