JCMS
OPEN ACCESS

www.jclinmedsurgery.com

Journal of
Clinical & Medical Surgery

Research Article

The Clinical Significance of Hippocalcin like-1 Protein in Glioblastoma Multiforme

Zihan Ran[1,2,5#]; Jiaxuan Bian[2#]; Jingcheng Yang[3,4,5]*

[1]Department of Research, Shanghai University of Medicine & Health Sciences Affiliated Zhoupu Hospital, 1500 Zhouyuan Road, Shanghai 201318, China.

[2]Inspection and Quarantine Department, The College of Medical Technology, Shanghai University of Medicine & Health Sciences, 279 Zhouzhu Road, Shanghai 201318, China.

[3]State Key Laboratory of Genetic Engineering, Human Phenome Institute, School of Life Sciences and Shanghai Cancer Center, Fudan University, 2005 Songhu Road, Shanghai 200438, China

[4]Greater Bay Area Institute of Precision Medicine, 115 Jiaoxi Road, Guangzhou 511458, China.

[5]The Genius Medicine Consortium (TGMC), 2005 Songhu Road, Shanghai, Shanghai, China.

***Corresponding Author: Jingcheng Yang**
State Key Laboratory of Genetic Engineering, Human Phenome Institute, School of Life Sciences and Shanghai Cancer Center, Fudan University, 2005 Songhu Road, Shanghai 200438, China
Email: yjcyxky@163.com

# These authors contributed equally to this work.

Article Info

Received: May 17, 2022
Accepted: Jun 03, 2022
Published: Jun 09, 2022
Archived: www.jclinmedsurgery.com

Abstract...

Background: Glioblastoma Multiforme (GBM) is a primary aggressive brain tumor that is difficult to treat. Due to a lack of effective diagnostic strategies, many patients have a suboptimal clinical status. Hippocalcin like-1 protein (HPCAL1) is a type of calcium sensor. Calcium ion (Ca+) is a crucial signaling factor in eukaryotic cells, which has a significant role in regulating the progression of GBM. This study aims to investigate the possibility of HPCAL1 as a diagnostic and prognostic biomarker for GBM through data analysis.

Methods: Clinical data from healthy individuals and GBM patients were retrospectively collected from databases such as Oncomine, Human Protein Atlas (HPA), Gene Expression Profile Interaction Analysis (GEPIA), and UALCAN. Cross-analysis were performed on these data, including expression analysis, survival analysis, methylation analysis, and PPI network analysis. Statistical significance was set at < 0.05.

Results: Data from GEPIA ($P < 0.05$) and UALCAN ($P=0.0166384$) showed significant differences in HPCAL1 expression between GBM and normal cells. In addition, data from GEPIA ($P = 0.013$), UALCAN ($P = 0.0025$) and HPA ($P=0.000057$) indicated that patients with higher HPCAL1 expression had a poorer prognosis. The gap junction in the KEGG pathway is associated with HPCAL1 and GBM.

Conclusion: The study showed that high expression of HPCAL1 in GBM cells was detrimental to patient survival. HPCAL1 could be used as a potential diagnostic and prognostic biomarker for GBM.

Citation: Ran Z, Bian J, Yang J. The Clinical Significance of Hippocalcin like-1 Protein in Glioblastoma Multiforme. J Clin Med Surgery. 2022; 2(1): 1014.

Introduction

As the most aggressive primary intracranial tumor, Glioblastoma (GBM) is challenging to treat, and the clinical prognosis of patients is poor [1]. Despite recent progress in cancer therapy, the treatment of GBM is still challenging [2-4]. Research studies have shown that the one-year survival rate for GBM patients is 36.5% [5], and the average survival time of GBM does not exceed 16 months [6,7]. Even numerous clinical trials have been conducted using different drugs, and they have not yet proven successful in terms of efficacy [8]. In addition, the current targeted therapies and immunotherapy are ineffective, so it is urgent to explore more new biomarkers to save more GBM patients in need [9].

Calcium ion (Ca^{2+}) has been recognized as an essential secondary signaling factor in eukaryotic cells for a long time [10]. Some reports indicate that calcium ions (Ca^{2+}) affect the progression of GBM by enhancing the quiescence, proliferation, migration, and maintenance of malignant cells, and thus could regard as an essential positive modulator [11]. In addition, studies have shown that increased Ca^{2+} degree in GBM improves the expression level of Hippocalcin like-1 protein (HPCAL1), and enhances GBM cell proliferation [12].

HPCAL1 is a neuron-specific calcium-binding protein found in the retina and brain [13]. Some studies have shown that HPCAL1 expression occurred mainly in Purkinje cells of the brain, and that HPCAL1 may be involved in the regulation of neuronal types [14,15]. It may participate in the calcium-dependent regulation of rhodopsin phosphorylation and is of relevance for neuronal signaling in the central nervous system [15,16]. In addition, it has been realized that HPCAL1 is an innovative inhibitor of liver cancer, which was downregulated in Hepatocellular Carcinoma (HCC) cells and tissues. Suppression of HPCAL1 expression is adverse to the clinical outcome of HCC patients [17].

In summary, the function of HPCAL1 is instructive for the study of its prognostic value and specific expression atlas in GBM. However, there are no reports in this regard yet. Therefore, we explored the expression, prognosis, methylation, and related co-expressed genes of HPCAL1 in multiple public databases in this study to determine the diagnostic and prognostic value of HPCAL1 in GBM. We present the following article in accordance with the REMARK reporting checklist

Materials and methods

Oncomine

Currently, Oncomine (https://www.oncomine.org) is the world's most fantastic integrated data-mining platform and oncogene microarray database designed to mine cancer genetic information. It has the perfect cancer mutation data, gene expression data, and relevant clinical information. It is essential in studies related to the analysis of new biomarkers or new therapeutic targets [18]. The expression of HPCAL1 mRNA was investigated in a range of cancers was compared with normal tissues. The following thresholds were used in this study: the gene rank, P-value, and fold change were 10%, 0.001, and 2, respectively.

Gene expression profiling interactive analysis (GEPIA)

GEPIA (http://gepia.cancer-pku.cn/index.html) is an interactive web-based analysis platform commonly used to analyze expression data from a total of 9736 tumors and 8587 standard specimens sequenced by RNA from the Genotype-Tissue Expression Project and The Cancer Genome Atlas (TCGA) [19]. This study analyzed the potential of HPCAL1 as a biomarker by analyzing data on differential expression levels and prognosis of HPCAL1 on the website. In all, we detected 163 GBM patients and 207 normal individuals. We selected co-expressed genes of HPCAL1 when PCC (Pearson correlation coefficient) was not less than 0.60 in the present study.

Human protein atlas (HPA)

The HPA database (https://www.proteinatlas.org/) is a Swedish program that was established in 2003. It is a public database of over 26,000 antibodies targeting more than 17,000 human genes for free [20,21]. We analyzed the prognosis and survival of 153 patients (High expression, n=41; Low expression, n=112) with glioma using the HPA database. We have chosen the best expression cut-off, 8.02.

UALCAN

UALCAN (http://ualcan.path.uab.edu/) is a comprehensive, user-friendly, and interactive platform for analyzing cancer OMICS data. It is often used for biomarker identification, expression profiling, survival analysis, gene methylation analysis, etc [22]. We analyzed HPCAL1 in UALCAN. The expression level (GBM, n = 156; normal, n = 5) and methylation status (GBM, n = 140; normal, n = 2) were evaluated.

STRING

The STRING database (https://string-db.org) is a web-based platform for known protein interactions [23], and it has been updated to version 11.5. In this study, we used the latest version of the database. By constructing a PPI network, we enriched and analyzed the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for HPCAL1 and its co-expressed genes. Some of these data were interpreted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) [24].

Statistical analysis

SPSS version 23.0 software was applied in this study for data analysis (IBM analysis). The research used Student's t-test to assay the differential expression of HPCAL1 between patients and normal. We used Pearson Correlation Coefficient (PCC) analysis to assess the association between HPCAL1 and its related genes. The survival outcomes were indicated with hazard ratios (HRs). Data were statistically significant when the P-value did not exceed 0.05.

Results

The expression level of HPCAL1 in GBM

This research evaluated the expression level of HPCAL1 mRNA in a range of cancers and normal through the Oncomine database firstly (Figure 1a). The research data have shown that HPCAL1 is significantly decreased in the brain and CNS Cancer, compared to normal tissues ($P < 0.05$). Meanwhile, this study tested the HPCAL1 expression level in various cancer cell lines among the HPA database (Figure 1b). And it was observed that in GBM cell lines, the expression was relatively low.

HPCAL1 mRNA expression level between GBM tissues and normal were analyzed by quantitative analysis in GEPIA. (GBM, n = 163; normal, n = 207; Figure 2a) and UALCAN (GBM, n = 156; normal, n = 5; Figure 2b). In these databases, we consistently found that the expression level of HPCAL1 in GBM was relatively low compared with normal tissues (GEPIA, P < 0.05; UALCAN, P = 1.663840E-02).

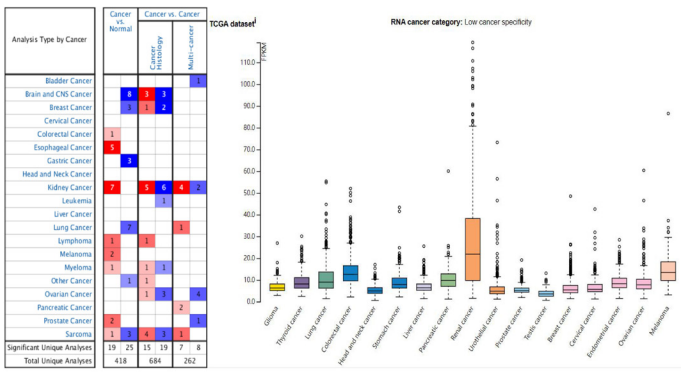
Methylation of HPCAL1 in GBM

We further analyzed the methylation changes of HPCAL1 in GBM using the UALCAN database (GBM, n=140; normal, n=2). However, there were no significant differences in the methylation degree of HPCAL in GBM compared to normal tissue (Figure 3a; P=0.90614).

Then, we further analyzed the methylation changes based on Age (Figure 3b), Gender (Figure 3c), and Race (Figure 3d), respectively. Although in general terms, no significant differences were found. However, we found statistically significant differences in some age categories (21-40 years vs 41-60 years, P=0.044035; 21-40 years vs 61-80 years, P=0.037738). There were statistically significant differences in a few groups, but we cannot exclude the sample's contingency. This situation may be worthy of further study.

Prognostic value of HPCAL1 in GBM

Afterward, the clinical data from healthy individuals and GBM patients was obtained from GEPIA, UALCAN, and HPA databases to analyze further the effects of different levels of HPCAL1 expression on the prognosis of patients (Figure 4). The results showed that HPCAL1 has a prognostic value in GBM, and high expression is detrimental to the prognosis of GBM patients (GEPIA, P = 0.013; UALCAN, P = 0.0025; HPA, P = 0.000057; Figure 4a-c). Subsequently, the expression level of HPCAL1 was analyzed in subgroups according to gender and race. The increasing trend of poor clinical outcomes HPCAL1 remains unchanged among female patients (Figure 4d, P=0.0087). While this phenomenon was not found in male patients. (Figure 4e, P=0.076). We also did not find any significance in other gender groups (P > 0.05). In Caucasian patients, the high expression level of HPCAL1 was closely tied with poor survival status (Figure 4f, P=0.0043). The rest did not differ significantly.

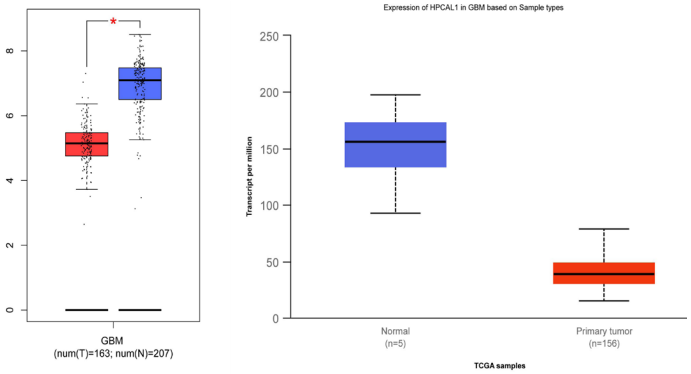


(a) (b)

Figure 1: The Expression of HPCAL1.
(a) Expression of HPCAL1 mRNA in various types of cancer. The numbers in the red and blue cells mean the number of analyses that meet the threshold. The shade of color is determined by the gene class. Red indicates over-expression of the gene, while blue indicates under-expression.
(b) Expression pattern of HPCAL1 mRNA in GBM cell lines from the HPA database.

Analysis of co-expressed genes associated with HPCAL1

We detected co-expressed genes associated with HPCAL1 through the GEPIA database. Two hundred genes were identified in total. When the cut-off correlation coefficient at 0.60, 12 similar expression patterns genes were obtained (Table 1). The PPI network was constructed on the STRING website to analyze the enriched functions in the network (Figure 5). We found that these genes are primarily expressed in the brain and are closely associated with ion channel regulatory activities. The analysis revealed the presence of a KEGG Pathways-gap junction (Table 2, TUBA4A, PRKCB, ITPR1) in these genes with similar expression patterns. We found that the Neuronal System (Table 3, STX1A, TUBA4A, PRKCB, KCNAB2, NRXN3) by Reactome Pathway analysis have an important position at the same time.



(a) (b)

Figure 2: The expression level of HPCAL1 mRNA in GEPIA (a) and UALCAN (b).

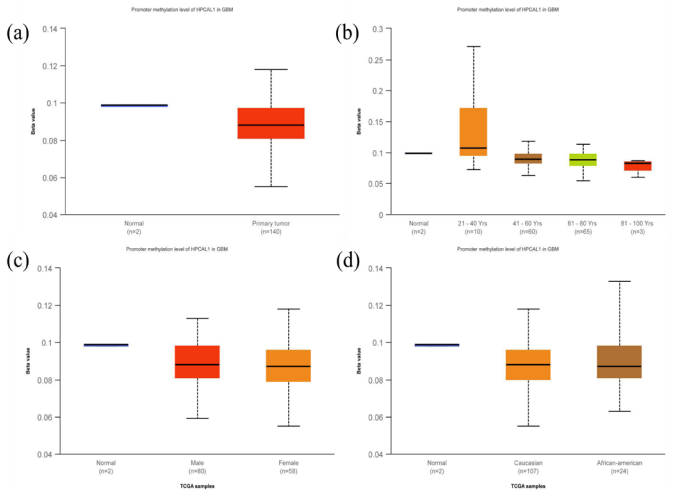


Figure 3: Assessing the methylation degree of HPCAL1 in GBM.
(a) HPCAL1 methylation between GBM tissues and normal.
(b) Age-based methylation analysis of HPCAL1.
(c) Sex-based methylation of HPCAL1.
(d) Race-based methylation of HPCAL1. Beta values indicate the methylation degree of DNA from 0 (unmethylated) to 1 (fully methylated). Differences in beta cut-off indicate hypermethylation (0.7-0.5) or hypomethylation (0.3-0.25).

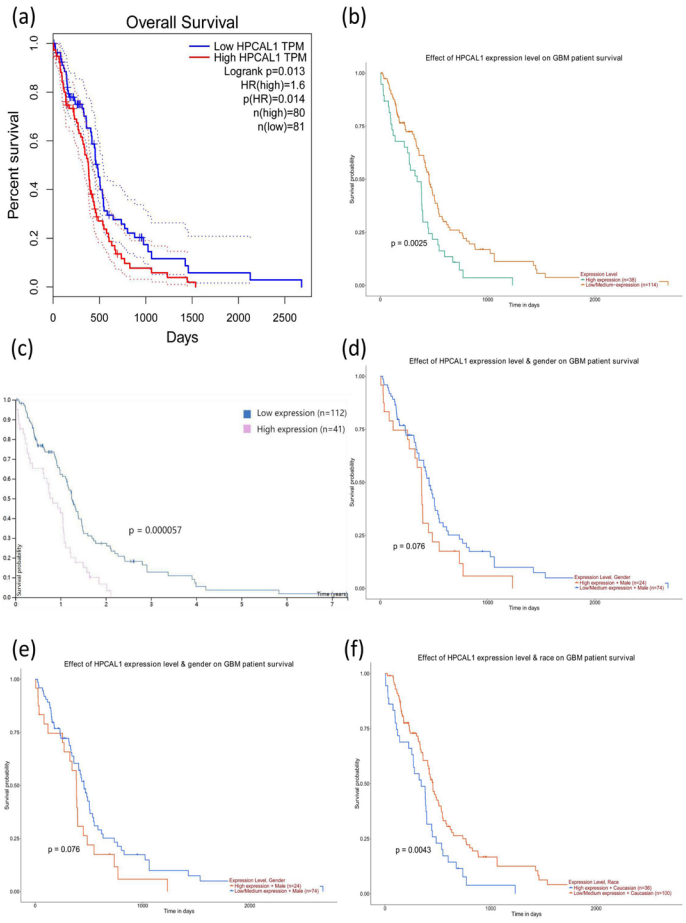


Figure 4: Prognostic value of HPCAL1 for GBM analyzed by GEPIA(a), UALCAN (b) and HPA (c). Subgroup survival analysis based on gender (d, e) and race (f).

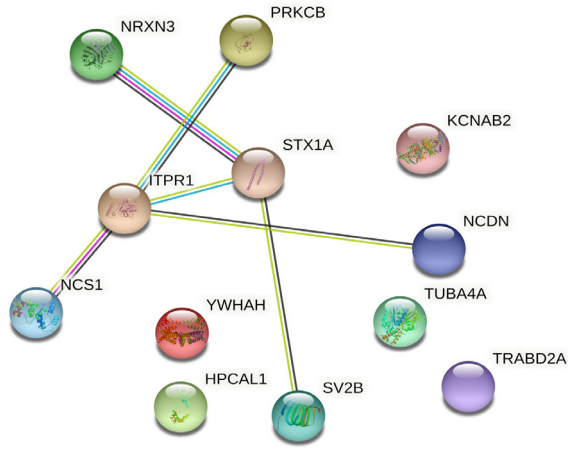


Figure 5: Establishing a PPI network to analyze the association between HPCAL1 and its genes with similar expression patterns.

Table 1: Genes with high similarity expression patterns to HPCAL1 were identified using 0.60 as the PCC intercept value.

Gene Symbol	Gene ID	PCC
TRABD2A	ENSG00000186854.10	0.67
STX1A	ENSG00000106089.11	0.65
KCNAB2	ENSG00000069424.14	0.64
ITPR1	ENSG00000150995.17	0.63
NCS1	ENSG00000107130.9	0.63
TUBA4A	ENSG00000127824.13	0.63
SV2B	ENSG00000185518.11	0.62
NCDN	ENSG00000020129.15	0.61
NRXN3	ENSG00000021645.17	0.61
YWHAH	ENSG00000128245.14	0.61
PRKCB	ENSG00000166501.12	0.60

PCC: Pearson Correlation Coefficient.

Table 2: KEGG pathways related to HPCAL1 co-expressed genes in GBM.

Term	Description	Gene count	False discovery rate	Matching proteins in network
Hsa04540	gap junction	3	0.0069	TUBA4A, PRKCB, ITPR1

KEGG: Kyoto encyclopedia of genes and genomes.

Table 3: Reactome pathways related to HPCAL1 co-expressed genes in GBM.

Term	Description	Gene count	False discovery rate	Matching proteins in network
HSA-112316	Neuronal System	5	0.0288	STX1A,TUBA4A,PRKCB, KCNAB2, NRXN3

Reactome: An open and open-source, peer-reviewed pathway database.

Discussion

The research summarized and analyzed data from various trustworthy databases and found the clinical characteristics of HPCAL1 in GBM patients. Compared with low-expressing HPCAL1, the high expression of HPCAL1 in GBM adversely affects the survival of patients, and the outcome was significantly different. However, no more significant differences were identified in the methylation levels of GBM patients. In addition, we analyzed the co-expressed genes of HPCAL1 and established a network analysis of these related genes, in which gap junction may play an important function.

HPCAL1 is a portion of the neuron-specific calcium-binding protein family. It is expressed in the brain and exists in granular cells and Purkinje cells [14,15]. HPCAL1 (VILIP-3) is also a member of the visinin-like (VSNL) protein family [25]. Reports are showing that the expression level of HPCAL1 is positively correlated with the intracellular Ca^{2+} concentration [12]. Ca^{2+} could play a vital regulatory role in the initiation of GBM by intervening in the maintenance, migration, quiescence, and proliferation of malignant cells [11]. Since the calcium pathway has been confirmed to be involved in regulating a variety of cellular reactions, it can be speculated that this pathway could impact the progression of malignant tumors [26].

HPCAL1 can act as a calcium downstream effector and have a proliferative effect on GBM cell proliferation [12]. It has also been shown that certain PHOX2B variants associated with neu-

roblastoma pathogenesis fail to bind to key interacting proteins such as HPCAL1, which may predispose to this malignancy by impeding the differentiation of immature sympathetic neurons [27]. HPCAL1 acts as an upstream factor of the Wnt/β-catenin axis [12], and the high expression of HPCAL1 has a promotional effect on GBM cell invasion and migration. These results echo the findings of our study, and the up-regulation of HPCAL1 in GBM cells was closely associated with worsening clinical outcomes in GBM patients.

We established a PPI network to enrich and analyze the KEGG pathway in HPCAL1 and its co-expressed genes. As a result, gap junctions were enriched. The gap junctions include intercellular channels, which allow direct communication between the cytosolic compartments of adjacent cells, and are one of the main modes of communication between neurons [28]. This type of intercellular communication permits the coordination of cellular activities and plays key roles in the control of cell growth and differentiation and in the maintenance of tissue homeostasis [29]. New evidence suggests that glial cells can secrete neuron-specific gap junction proteins such as Connexin43 [30], or miRNAs [31] that act as a bridge between glial cell bundles and neurons. And drugs targeting connexins will be a new option for the treatment of gliomas [32]. The network of glial cells through gap junctions plays a significant role in the entire brain circuit: they integrate neuronal signals, release calcium ions and excite to transmit information [33,34]. It has also been suggested that currents were detected at such gap junctions, indicating that such gap junctions are also involved in the process of nerve element differentiation [35]. In summary, we speculate that gap junctions may be bound up with the occurrence, development, and progress of GBM.

TUBA4A and PRKCB were found to be associated with HPCAL1 in both KEGG and Reactome Pathway analysis. Among them, TUBA4A is usually considered as a protein involved in ALS-related molecular pathways which include mitochondrial dysfunction, altered RNA metabolism, impaired cytoskeletal integrity, altered axonal transport dynamics, and DNA damage accumulation due to defective DNA repair [36]. PRKCB, a member of the classical PRKCs, negatively modulates the mitochondrial energy status and inhibits autophagy [37]. By understanding the specific roles of these associated genes, it may provide the link between HPCAL1 expression, which drives the migratory spread of GBM, and its upstream factor calcium ion (Ca+). We hope that these studies will provide further insights into the occurrence, development, and treatment of GBM.

In this study, the effect of different expression levels of HPCAL1 on the prognosis of GBM patients was collected and analyzed retrospectively. The results showed that high expression of HPCAL1 has a poor clinical outcome for GBM patients, due to its function of promoting GBM metastasis and invasion [12]. The results of this study may provide a new idea for further identification of GBM biomarkers in the future

Conclusions

HPCAL1 functions as a promoter of GBM metastasis and invasion, and its high expression is associated with poor clinical outcomes in GBM patients. HPCAL1 may serve as a potential diagnostic and prognostic biomarker for GBM

Declarations

Contributions:

(I) Conception and design: Zihan Ran & Jingcheng Yang;

(II) Administrative support: Zihan Ran & Jingcheng Yang;

(III) Provision of study materials or patients: Zihan Ran & Jiaxuan Bian;

(IV) Collection and assembly of data: Zihan Ran & Jiaxuan Bian;

(V) Data analysis and interpretation: Zihan Ran & Jiaxuan Bian;

(VI) Manuscript writing: All authors

(VII) Final approval of manuscript: All authors

Acknowledgements: The authors would like to thank The Genius Medicine Consortium (TGMC) provided computer cluster. This work was supported by Shanghai Sailing Program (21YF1418700); Shanghai Municipal Education Commission (ZZSHJKYXY20008); and Shanghai University of Medicine & Health Sciences (SSF-21-05-008 and A3-0200-21-311007-33).

Reporting checklist: The authors have completed the REMARK reporting checklist.

Conflicts of interest: All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

Ethical statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Data availability statement: The datasets involved in this research are available in the following databases: the ONCOMINE database, https://www.oncomine.org; the Gene Expression Profiling Interactive Analysis database(GEPIA), http://gepia.cancer-pku.cn/index.html; the Human Protein Atlas database(HPA), https://www.proteinatlas.org/; the UALCAN database, http://ualcan.path.uab.edu/ ,and the STRING database, https://string-db.org.

Keywords: Biomarkers; Inflammation; Lipids; Body fat; Cryolipolysis; Bio impedance.

Abbreviations: GBM: Glioblastoma Multiforme; HPCAL1: Hippocalcin Like-1 Protein; Ca+: Calcium Ion; HPA: Human Protein Atlas; GEPIA: Gene Expression Profile Interaction Analysis; HCC: Hepatocellular Carcinoma; TCGA: The Cancer Genome Atlas; KEGG: Kyoto Encyclopedia Of Genes And Genomes; Hrs: Hazard Ratios; PCC: Pearson Correlation Coefficient.

References

1. Quan R, Zhang H, Li Z, Li X. Survival analysis of patients with glioblastoma treated by long-term administration of temozolomide. Medicine (Baltimore). 2020; 99: e18591.

2. Estevez-Ordonez D, Chagoya G, Salehani A, Atchley TJ, Laskay NMB, Parr MS, et al. Immunovirotherapy for the Treatment of Glioblastoma and Other Malignant Gliomas. Neurosurg Clin N Am. 2021; 32: 265–81.

3. Tang W, Fan W, Lau J, Deng L, Shen Z, Chen X. Emerging blood-

brain-barrier-crossing nanotechnology for brain cancer theranostics. Chem Soc Rev. The Royal Society of Chemistry. 2019; 48: 2967–3014.

4. Yin L, Li H, Liu W, Yao Z, Cheng Z, et al. A highly potent CDK4/6 inhibitor was rationally designed to overcome blood brain barrier in gliobastoma therapy. Eur J Med Chem. 2018; 144: 1–28.

5. Ostrom QT, Gittleman H, Liao P, Vecchione-Koval T, Wolinsky Y, Kruchko C, et al. CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2010–2014. Neuro-Oncology. 2017; 19: v1–v88.

6. Yerukala Sathipati S, Huang H-L, Ho S-Y. Estimating survival time of patients with glioblastoma multiforme and characterization of the identified microRNA signatures. BMC Genomics. BioMed Central; 2016; 17: 1022–86.

7. Xia Y, Yang C, Hu N, Yang Z, He X, et al. Exploring the key genes and signaling transduction pathways related to the survival time of glioblastoma multiforme patients by a novel survival analysis model. BMC Genomics. BioMed Central; 2017; 18: 950–11.

8. Bruinsmann FA, Richter Vaz G, de Cristo Soares Alves A, Aguirre T, Raffin Pohlmann A, Stanisçuaski Guterres S, et al. Nasal Drug Delivery of Anticancer Drugs for the Treatment of Glioblastoma: Preclinical and Clinical Trials. Molecules. Multidisciplinary Digital Publishing Institute. 2019; 24: 4312.

9. Jue TR, McDonald KL. The challenges associated with molecular targeted therapies for glioblastoma. Journal of Neuro-Oncology. Springer US. 2016; 127: 427–34.

10. Zheng J, Zeng X, Wang S. Calcium ion as cellular messenger. Sci China Life Sci. Science China Press. 2015; 58: 1–5.

11. Leclerc C, Haeich J, Aulestia FJ, Kilhoffer M-C, Miller AL, Néant I, et al. Calcium signaling orchestrates glioblastoma development: Facts and conjunctures. Biochim Biophys Acta. 2016 Jun;1863(6 Pt B):1447–59.

12. Zhang D, Liu X, Xu X, Xu J, Yi Z, et al. HPCAL1 promotes glioblastoma proliferation via activation of Wnt/β-catenin signalling pathway. J Cell Mol Med. John Wiley & Sons, Ltd. 2019; 23: 3108–17.

13. Burgoyne RD. Neuronal calcium sensor proteins: generating diversity in neuronal Ca2+ signalling. Nat Rev Neurosci. Nature Publishing Group. 2007; 8: 182–93.

14. Mahloogi H, González-Guerrico AM, Lopez De Cicco R, Bassi DE, Goodrow T, et al. Overexpression of the calcium sensor visinin-like protein-1 leads to a cAMP-mediated decrease of in vivo and in vitro growth and invasiveness of squamous cell carcinoma cells. Cancer Res. Cancer Res. 2003; 63: 4997–5004.

15. Spilker C, Gundelfinger ED, Braunewell K-H. Evidence for different functional properties of the neuronal calcium sensor proteins VILIP-1 and VILIP-3: from subcellular localization to cellular function. Biochim Biophys Acta. 2002; 1600: 118–27.

16. Burgoyne RD, Weiss JL. The neuronal calcium sensor family of Ca2+-binding proteins. Biochem J. Portland Press Ltd. 2001; 353: 1–12.

17. Zhang Y, Liu Y, Duan J, Yan H, Zhang J, Zhang H, et al. Hippocalcin-like 1 suppresses hepatocellular carcinoma progression by promoting p21(Waf/Cip1) stabilization by activating the ERK1/2-MAPK pathway. Hepatology. John Wiley & Sons, Ltd. 2016; 63: 880–97.

18. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia. 2007; 9: 166–80.

19. Tang Z, Li C, Kang B, Gao G, Li C, ET AL. GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Research. 2017; 45: W98–W102.

20. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, et al. Proteomics. Tissue-based map of the human proteome. Science. American Association for the Advancement of Science. 2015; 347: 1260419.

21. Uhlén M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, et al. Towards a knowledge-based Human Protein Atlas. Nat Biotechnol. Nature Publishing Group; 2010; 28: 1248–50.

22. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia. 2017; 19: 649–58.

23. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Research. 2021 Jan 8; 49: D605–12.

24. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Research. 2000; 28: 27–30.

25. Rebaud S, Simon A, Wang CK, Mason L, Blum L, et al. Comparison of VILIP-1 and VILIP-3 binding to phospholipid monolayers. PLoS ONE. Public Library of Science; 2014; 9: e93948.

26. Roderick HL, Cook SJ. Ca2+ signalling checkpoints in cancer: remodelling Ca2+ for cancer cell proliferation and survival. Nat Rev Cancer. Nature Publishing Group; 2008; 8: 361–75.

27. Wang W, Zhong Q, Teng L, Bhatnagar N, Sharma B, et al. Mutations that disrupt PHOXB interaction with the neuronal calcium sensor HPCAL1 impede cellular differentiation in neuroblastoma. Oncogene. Nature Publishing Group. 2014; 33: 3316–24.

28. Nielsen MS, Axelsen LN, Sorgen PL, Verma V, Delmar M, ET AL. Gap junctions. Compr Physiol. John Wiley & Sons, Ltd; 2012; 2: 1981–2035.

29. Aasen T, Leithe E, Graham SV, Kameritsch P, Mayán MD, et al. Connexins in cancer: bridging the gap to the clinic. Oncogene. Nature Publishing Group. 2019; 38: 4429–51.

30. Chepied A, Daoud-Omar Z, Meunier-Balandre A-C, Laird DW, Mesnil M, et al. Involvement of the Gap Junction Protein, Connexin43, in the Formation and Function of Invadopodia in the Human U251 Glioblastoma Cell Line. Cells. Multidisciplinary Digital Publishing Institute. 2020; 9: 117.

31. Peng Y, Wang X, Guo Y, Peng F, Zheng N, et al. Pattern of cell-to-cell transfer of microRNA by gap junction and its effect on the proliferation of glioma cells. Cancer Sci. John Wiley & Sons, Ltd; 2019; 110: 1947–58.

32. Mulkearns-Hubert EE, Torre-Healy LA, Silver DJ, Eurich JT, Bayik D, Serbinowski E, et al. Development of a Cx46 Targeting Strategy for Cancer Stem Cells. Cell Rep. 2019; 27: 1062–5.

33. Araque A, Navarrete M. Glial cells in neuronal network function. Philos Trans R Soc Lond B Biol Sci. The Royal Society. 2010; 365: 2375–81.

34. Belousov AB, Fontes JD. Neuronal gap junctions: making and breaking connections during development and injury. Trends Neurosci. 2013; 36: 227–36.

35. Jabeen S, Thirumalai V. The interplay between electrical and chemical synaptogenesis. J Neurophysiol. American Physiological Society Bethesda, MD. 2018; 120: 1914–22.

36. Chia R, Chiò A, Traynor BJ. Novel genes associated with amyotrophic lateral sclerosis: diagnostic and clinical implications. Lancet Neurol. 2018; 17: 94–102.

37. Patergnani S, Marchi S, Rimessi A, Bonora M, Giorgi C, et al. PRKCB/protein kinase C, beta and the mitochondrial axis as key regulators of autophagy. Autophagy. Taylor & Francis. 2013; 9: 1367–85.