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ORIGINAL ARTICLE

Cell Division Cycle-associated 7-like Gene: A Novel Biomarker for Adverse Survival in Human High-grade Gliomas

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Background: High-grade primary gliomas are aggressively growing and have an unfavorable prognosis. The utility of prognostic biomarkers of outcome in glioma patients is important for medical practice. Cell division cycle-associated 7-like (CDCA7L) protein modifies cancer progression and metastasis. Nevertheless, its character in defining the clinical prognosis of human gliomas has not been illuminated. **Subjects and Methods:** The hypothesis of this study was that CDCA7L is upregulated in human gliomas. We studied two de-linked data from Gene Expression Omnibus (GEO) profile. The first dataset (GDS1816/225081_s_at/CDCA7L) in primary high-grade glioma included age, gender, and survival time. Another dataset (GDS1962/225081_s_at/CDCA7L) was also encompassed to estimate CDCA7L gene expression in each pathological grading. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) was used to survey the protein–protein interaction (PPI) network of CDCA7L-regulated oncogenesis. **Results:** Statistical analysis of the GEO profile revealed that the World Health Organization (WHO) Grade IV (n = 81) gliomas had higher CDCA7L mRNA expression level than in Grade II (n = 7, $P = 2.15 \times 10^{-14}$) gliomas and nontumor controls (n = 23, $P = 2.87 \times 10^{-18}$). Kaplan–Meier analysis reported that patients with high CDCA7L mRNA levels (n = 49) had adverse survival than those with low CDCA7L expression (n = 28). The PPI analysis of CDCA7L-regulated oncogenesis showed CDCA7L as a potential hub protein. **Conclusions:** The expression of CDCA7L has a positive correlation with the WHO pathological grading and shorter survival. This finding suggests that CDCA7L may be a potential biomarker of prognosis in human gliomas.

Key words: Cell division cycle-associated 7-like, gene expression omnibus profile, glioma, World Health Organization, pathological grades

INTRODUCTION

In adults, glioblastomas are the most common primary brain tumors and have a poor response to current treatment. The median survival time is only 14.6 months even the patients underwent aggressive surgical resection and chemo-radiotherapy.[1] The World Health Organization (WHO) classification defined the pathological grading of gliomas.[2] High-grade gliomas have a poor prognosis and shorter survival. Therefore, biomarkers for

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the prediction of clinical outcome are mandatory. Using genetic biomarker to distinguish the genetic expression in brain tumors is important for clinical practice to improve outcome.[3]

Cell division cycle-associated 7-like (CDCA7L) gene is one of the c-Myc-regulated genes.[4] c-Myc colocalizes and interacts with CDCA7L in the nucleus to enforce CDCA7L gene expression.[4,5] Moreover, CDCA7L could complement the c-Myc transformation-defective mutant in Rat1a fibroblast cell line and increase Myc-mediated transformation.[4] Recently, CDCA7L overexpression is recognized in medulloblastoma cells,[4] breast cancer,[6] and hepatocellular carcinoma.[7] However, its role in deciding the pathological grading and survival of human gliomas has not been addressed.

This study is designed to determine whether CDCA7L expression associates with the survival and WHO pathological grading of human gliomas under the assumption that high-grade brain tumors having elevated CDCA7L expression. The dataset from Gene Expression Omnibus (GEO) profile offers wide genetic analyses of human gene expression and disease connections.[8,9] Statistical analyses of the dataset from GEO profiles disclose that expression of CDCA7L confidently associates with the WHO grading and poor outcome in primary glioma patients. All these data propose that CDCA7L might be a prognostic biomarker in human gliomas.

PATIENTS AND METHODS

Cell division cycle-associated 7-like gene expression in human gliomas

The Institutional Review Board (TSGHIRB No: B-102-10) approved the study in Tri-Service General Hospital, Taipei, Taiwan, ROC. Former studies had designated the procedure for the analyses of functional genomic databases.[10,11] The first dataset (GDS1816/225081_s_at/CDCA7L) contains 100 sheets of de-linked data, including CDCA7L mRNA expression, sex, age, and pathological grading of primary high-grade glioma which were acquired from http://www.ncbi.nlm.nih.gov/geoprofiles/?term = GDS1816+%2F + 225081_s_at+%2F + CDCA7L. After omitting 23 sheets of data without thorough information on survival times and age, 77 sheets were enrolled in the statistical analyses.

An extra database (GDS1962/225081_s_at/CDCA7L) including 180 sheets from 23 patients without tumor (i.e. nontumor control), seven with Grade II glioma, 19 with Grade III glioma, and 81 with Grade IV glioma on CDCA7L gene expression acquired from http://www.ncbi.nlm.nih.gov/geoprofiles/?term = GDS1962+%2F + 225081_s_at+%2F + CDCA7L was also encompassed. Since the genetic contribution to the prognosis of oligodendroglioma is dramatically different from gliomas, 38 sheets with Grade II ODG and 12 sheets with Grade III ODG were excluded from the analyses.

Statistical analysis of cell division cycle-associated 7-like gene expression in human high-grade gliomas

Single tail *t*-test was used for analyzing the value of CDCA7L expression from dataset (GDS1962/225081_s_at/CDCA7L) in the four pathological grades. The R 3.0.1 software (R Foundation for Statistical Computing, Vienna, Austria) package of Bonferroni method was used to adjust the *P* value to eliminate the risk of type I error in multi-group analyses.

The overall survival analysis and cohorts of low versus high-CDCA7L expressions in high-grade gliomas from the GEO profile (GDS1816/225081_s_at/CDCA7L) were revealed via Kaplan–Meier method. The cutoff point of CDCA7L expression was presented as median value that was decided by statistical analysis. Analyses of survival variables in the dataset (GDS1816/225081_s_at/CDCA7L) of high-grade glioma patients, including the WHO Grade III combined with Grade IV human glioma groups, were executed by Chi-square test. The GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA) was used to generate the figures, and $P < 0.05$ was considered statistically significant.

Protein–protein interactions network and signaling pathways analysis

The methodology for known and predicted protein–protein interactions (PPIs) was described previously.[11] We analyzed the signaling pathways by applying Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, version 10.0 (http://string-db.org).[12]

RESULTS

Cell division cycle-associated 7-like mRNA expression confidently correlated with the World Health Organization pathological grading of human gliomas

As shown in Figure 1, the CDCA7L expression level was higher in the WHO Grade IV ($n = 81$) than in Grade II gliomas ($n = 7$; $P = 2.15 \times 10^{-14}$) and nontumor controls ($n = 23$) ($P = 2.87 \times 10^{-18}$, *P* adjusted by Bonferroni method). Our data established that high-grade gliomas had CDCA7L overexpression.

High cell division cycle-associated 7-like mRNA expression correlated with a poor survival in high-grade human gliomas

As shown in Figure 2, the Kaplan–Meier survival analysis found that high CDCA7L mRNA expression displayed a poor overall survival in high-grade glioma patients ($P < 0.0001$, by

log-rank test; 95% confidence interval: 2.126–5.299, hazard ratio 2.66). The cutoff value was set at 1550.2. The median survival in the high- and low-CDCA7L expression groups was 62 weeks and 189 weeks, respectively.

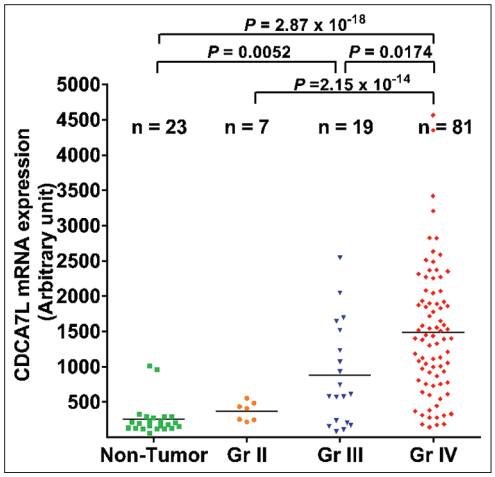

Figure 1: Cell division cycle-associated 7-like mRNA expression in nontumor control groups and glioma. The scattered plots revealed that the cell division cycle-associated 7-like mRNA gene expression in high-grade gliomas (Grades III and IV) was higher than in low-grade glioma and nontumor controls. Elevated cell division cycle-associated 7-like mRNA levels significantly correlated with the World Health Organization pathological grading of gliomas. The adjusted P value was estimated between each group

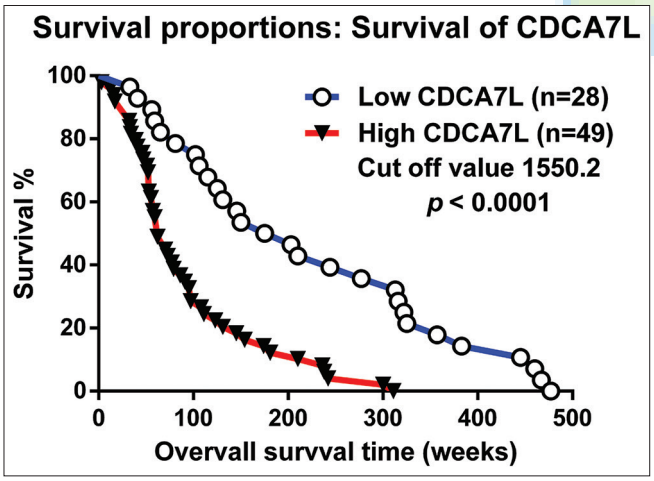

Figure 2: Overall survival in individuals with high-grade gliomas. The Kaplan–Meier survival curve disclosed unfavorable survival in those with high cell division cycle-associated 7-like expression (≤1550.2; n = 49) compared to those with low cell division cycle-associated 7-like expression (>1550.2; n = 28) with statistical significance (P = 0.0001, by log-rank test; 95% confidence interval: 2.126-5.299, hazard ratio 2.66). The median survival in the high- and low-cell division cycle-associated 7-like expression groups was 62 weeks and 189 weeks, respectively

DISCUSSION

The current study validates that CDCA7L expression was meaningfully higher in high-grade glioma patients than in nontumor controls and in low-grade glioma patients. In addition, glioma patients with high CDCA7L expression forecasted an unfavorable survival as compared to those with low CDCA7L expression.

The overexpression or amplification of c-Myc mRNA and proteins in glioma has been identified.[13-15] Previous study demonstrated that inhibition of c-Myc will subdue tumors by increasing differentiation of the glioma cells.[16] Therefore, c-Myc is associated with the regulation and, in particular, the proliferation of glioma stem cells, which are the theoretical cells of derivation for these brain tumors[16-18] CDCA7L belongs to the JPO family which is also called CDCA protein family. CDCA7L and its analog, CDCA7, both are recognized as c-Myc responsive genes and associated with malignancies.[7,19-21] CDCA7 and CDCA7L could interact with c-Myc and complement and increase Myc-mediated transformation, and could consequently further join neoplastic transformation and lead to tumorigenesis.[4,19-22]

The overexpression of CDCA7L is recognized in medulloblastoma cells, breast cancer, and hepatocellular carcinoma.[4,6,7] CDCA7L overexpression will cause cell cycle dysregulation and contribute to tumorigenesis. Huang *et al*. found that CDCA7L plays a crucial role in medulloblastoma tumor development evidenced by increasing colony formation and performs Myc-mediated transformation of medulloblastoma cells.[4] Another study reported that CDCA7L overexpression raises the progression of hepatocellular carcinoma cell cycle from G0/G1 phase into S phase while CDCA7L knockdown overturns tumorigenicity and decreases tumor size *in vivo*.[7] Further study unveiled that CDCA7L could stimulate the extracellular signal-regulated kinase 1/2 signaling pathway and upregulate cyclin D1 expression.[7] Moreover, CDCA7L is encompassed in apoptotic signaling pathway because CDCA7L is a suppressor of monoamine oxidase A (MAOA) via interaction with MAOA's core promoter, Sp1-binbding sites.[5,23] The inhibition of MAOA will result in lessened apoptosis through elevated expression of the cell cycle enhancers E2F1 and cyclin D1.[5,24,25] We used STRING to survey the PPI network of CDCA7L-regulated oncogenesis.[12] The network disclosed three protein clusters connected by CDCA7L as a potential hub protein [Figure 3].

This study's restriction included difficulty in gathering big numbers of human gliomas for investigating CDCA7L mRNA

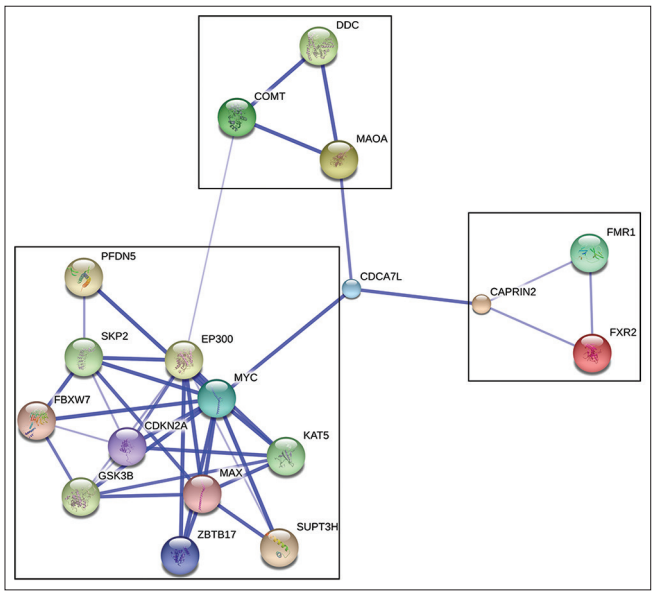

Figure 3: The cell division cycle-associated 7-like protein–protein interaction network. The PPI network of cell division cycle-associated 7-like was created using Search Tool for the Retrieval of Interacting Genes/Proteins database and presented in the confidence view. The network discloses that cell division cycle-associated 7-like protein is a hub protein for three protein clusters, as shown by black squares, linked by cell division cycle-associated 7-like protein

expression or protein level, predominantly Grade II low-grade gliomas or nontumor controls. Instead, statistical analyses of two GEO profiles with total 208 sheets were used to define the role of CDCA7L as a pathological grading and prognostic biomarker of unfavorable outcome.[9,26]

CONCLUSIONS

The study result revealed that CDCA7L expression has a meaningful correlation with different WHO pathological grades of human gliomas. Overexpression of CDCA7L is associated with an unfavorable survival in high-grade gliomas. Therefore, CDCA7L is correlated with pathological grading of human gliomas and may be a potential survival biomarker.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
2. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, *et al.* The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007;114:97-109.
3. Roversi G, Pfundt R, Moroni RF, Magnani I, van Reijmersdal S, Pollo B, *et al.* Identification of novel genomic markers related to progression to glioblastoma through genomic profiling of 25 primary glioma cell lines. Oncogene 2006;25:1571-83.
4. Huang A, Ho CS, Ponzielli R, Barsyte-Lovejoy D, Bouffet E, Picard D, *et al.* Identification of a novel c-Myc protein interactor, JPO2, with transforming activity in medulloblastoma cells. Cancer Res 2005;65:5607-19.
5. Ou XM, Chen K, Shih JC. Monoamine oxidase A and repressor R1 are involved in apoptotic signaling pathway. Proc Natl Acad Sci U S A 2006;103:10923-8.
6. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, *et al.* Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530-6.
7. Tian Y, Huang C, Zhang H, Ni Q, Han S, Wang D, *et al.* CDCA7L promotes hepatocellular carcinoma progression by regulating the cell cycle. Int J Oncol 2013;43:2082-90.
8. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, *et al.* NCBI GEO: Archive for functional genomics data sets – update. Nucleic Acids Res 2013;41:D991-5.
9. Barrett T, Troup DB, Wilhite SE, Ledoux P, Rudnev D, Evangelista C, *et al.* NCBI GEO: Archive for high-throughput functional genomic data. Nucleic Acids Res 2009;37:D885-90.
10. Tsai WC, Chen Y, Huang LC, Lee HS, Ma HI, Huang SM, *et al.* EMMPRIN expression positively correlates with WHO grades of astrocytomas and meningiomas. J Neurooncol 2013;114:281-90.
11. Hueng DY, Tsai WC, Chiou HY, Feng SW, Lin C, Li YF, *et al.* DDX3X biomarker correlates with poor survival in human gliomas. Int J Mol Sci 2015;16:15578-91.
12. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, *et al.* STRING v10: Protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015;43:D447-52.
13. Herms JW, von Loewenich FD, Behnke J, Markakis E,

Kretzschmar HA. c-myc oncogene family expression in glioblastoma and survival. Surg Neurol 1999;51:536-42.
14. Faria MH, Khayat AS, Burbano RR, Rabenhorst SH. c-MYC amplification and expression in astrocytic tumors. Acta Neuropathol 2008;116:87-95.
15. Hodgson JG, Yeh RF, Ray A, Wang NJ, Smirnov I, Yu M, *et al.* Comparative analyses of gene copy number and mRNA expression in glioblastoma multiforme tumors and xenografts. Neuro Oncol 2009;11:477-87.
16. Zheng H, Ying H, Yan H, Kimmelman AC, Hiller DJ, Chen AJ, *et al.* p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. Nature 2008;455:1129-33.
17. Broaddus WC, Chen ZJ, Prabhu SS, Loudon WG, Gillies GT, Phillips LL, *et al.* Antiproliferative effect of c-myc antisense phosphorothioate oligodeoxynucleotides in malignant glioma cells. Neurosurgery 1997;41:908-15.
18. Wang J, Wang H, Li Z, Wu Q, Lathia JD, McLendon RE, *et al.* c-Myc is required for maintenance of glioma cancer stem cells. PLoS One 2008;3:e3769.
19. Prescott JE, Osthus RC, Lee LA, Lewis BC, Shim H, Barrett JF, *et al.* A novel c-Myc-responsive gene, JPO1, participates in neoplastic transformation. J Biol Chem 2001;276:48276-84.
20. Goto Y, Hayashi R, Muramatsu T, Ogawa H, Eguchi I, Oshida Y, *et al.* JPO1/CDCA7, a novel transcription factor E2F1-induced protein, possesses intrinsic transcriptional regulator activity. Biochim Biophys Acta 2006;1759:60-8.
21. Osthus RC, Karim B, Prescott JE, Smith BD, McDevitt M, Huso DL, *et al.* The myc target gene JPO1/CDCA7 is frequently overexpressed in human tumors and has limited transforming activity *in vivo*. Cancer Res 2005;65:5620-7.
22. Gill RM, Gabor TV, Couzens AL, Scheid MP. The MYC-associated protein CDCA7 is phosphorylated by AKT to regulate MYC-dependent apoptosis and transformation. Mol Cell Biol 2013;33:498-513.
23. Ou XM, Chen K, Shih JC. Glucocorticoid and androgen activation of monoamine oxidase A is regulated differently by R1 and Sp1. J Biol Chem 2006;281:21512-25.
24. Chen K, Ou XM, Chen G, Choi SH, Shih JC. R1, a novel repressor of the human monoamine oxidase A. J Biol Chem 2005;280:11552-9.
25. Johnson S, Stockmeier CA, Meyer JH, Austin MC, Albert PR, Wang J, *et al.* The reduction of R1, a novel repressor protein for monoamine oxidase A, in major depressive disorder. Neuropsychopharmacology 2011;36:2139-48.
26. Hueng DY, Lin GJ, Huang SH, Liu LW, Ju DT, Chen YW, *et al.* Inhibition of Nodal suppresses angiogenesis and growth of human gliomas. J Neurooncol 2011;104:21-31.