Original Article

Epithelial cell transformation sequence 2 is a potential biomarker of unfavorable survival in human gliomas

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Abstract

Background: High-grade primary gliomas are invasive and have poor outcome. The identification of biomarkers predictive of outcome in patients with gliomas is crucial for clinical follow-up. Epithelial cell transformation sequence 2 (ECT2) modulates cancer invasion, progression, metastasis and cell cycle regulation. However, its role in determining the clinical outcome of human gliomas warrants further elucidation. **Materials and Methods:** This study hypothesized that ECT2 is over-expressed in human gliomas. We analysis de-linked data (GDS1815/219787_s_at/ECT2) in primary high-grade glioma, and exclude 23 sheets of data without detailed information. An additional database (GDS1962/234992_x_at/ECT2) was also included to evaluation ECT2 gene expression in each pathologic grading. **Results:** Analysis of the Gene Expression Omnibus (GEO) profile showed that ECT2 mRNA expression level was higher in WHO grade IV ($n = 81$) than in grade II ($n = 7$, $P = 0.0126$) gliomas and non-tumor controls ($n = 23$; $P = 1.65 \times 10^{-8}$). Kaplan-Meier analysis showed unfavorable survival in patients with high ECT2 mRNA levels ($n = 10$) than in those with low ECT2 expression ($n = 67$) (median survival, 106 vs. 46 weeks, $P < 0.0001$, by log-rank test, Hazard ratio: 0.07850, 95% CI: 0.02402–0.2565). **Conclusions:** ECT2 expression is positively correlated with WHO pathologic grading and unfavorable survival, suggesting that ECT2 may be a potential therapeutic candidate in human gliomas.

Key words: *Epithelial cell transformation sequence 2, gene expression omnibus profile, glioma, World Health Organization pathologic grades*

Introduction

Glioblastomas are common brain tumors with poor prognosis because of their invasive feature despite aggressive surgical resection and chemo-radiotherapy.[1]

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The World Health Organization (WHO) classification defined the pathologic grading of gliomas.[2] For instance, high-grade gliomas have poor prognosis and high mortality. Because clinical outcome can be predicted by some biomarkers, using genetic biomarker to distinguish the genetic expression in brain tumors is important for improving outcome.[3]

The Rho family of GTPases is the main regulator of glioma migration and invasion.[4] Epithelial cell transforming sequence 2 (ECT2), a 104 kDa protein, plays the role of guanine nucleotide exchange factor for the Rho family GTPase.[5] ECT2 modulates cancer invasion, progression, and metastasis.[6] Its over-expression is identified in

many human malignancies,[7] including cancers of the breast,[8,9] lungs,[10-12] esophagus,[10,13] liver, pancreas,[14,15] ovaries,[16,17] bladder,[16] and brain.[18-20] However, its role in determining the pathologic grading and survival of human gliomas[19] is less addressed.

Under the hypothesis that high-grade brain tumors over-express ECT2 level, this study aimed to determine if ECT2 expression correlates with the WHO pathologic grading and survival in human gliomas. The Gene Expression Omnibus (GEO) profiles offer a dataset of broad genetic analyses of human gene expression and disease correlations.[21,22] Analyses of the database from GEO profiles reveal that ECT2 expressions positively correlate with WHO grading and unfavorable outcome in patients with primary gliomas, suggesting that ECT2 could may be a predictive biomarker in human gliomas.

Materials and Methods

Study ethics and ECT2 gene expression in human brain tumor

The institutional review board of Tri-Service General Hospital in Taipei, Taiwan, ROC approved the study (TSGHIRB No: B-102-10). The methodology for analyses of functional genomic databases was as described previously.[23] Briefly, 100 sheets of de-linked data (GDS1815/219787_s_at/ECT2) on ECT2 mRNA expression, sex, age, and pathologic grading of primary high-grade glioma were obtained from http://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID = GDS1815:219787_s_at. Twenty-three sheets of data without detailed information on age and survival times were excluded so that 77 sheet were included in the statistical analyses.

An additional database (GDS1962/234992_x_at/ECT2) containing 180 sheets from 23 patients without tumor, seven with grade II glioma, 19 with grade III glioma, 81 with grade IV glioma, 38 with grade II oligodendroglioma and 12 with grade III oligodendroglioma with data on ECT2 gene expression obtained from http://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID = GDS1962:234992_x_at was also included.

Statistical analysis

Hybrid strategies were used to analyze the two datasets (GDS1815/219787_s_at/ECT2 and GDS1962/234992_x_at/ECT2) obtained from the GEO profiles. The values of ECT2 expression in the four pathologic grades were analyzed by single tail test. The Bonferroni method was used to adjust the *P* value to exclude the possibility of type I error in multi-groups analyses. The Kaplan-Meier method was used for the overall survival analysis and cohorts of high- versus low-ECT2 expressions were compared in high-grade gliomas. Chi-square test was performed to analyze survival variables in WHO grade III alone, and the patient populations from WHO grade III combined with grade IV human glioma groups. The cut-off point of ECT2 expression was decided by statistical analysis to obtain the adequate value. Statistical calculations were performed using the R 3.0.1 software. The figures were generated by the GraphPad Prism 5 software. Statistical significance was set at $P < 0.05$.

Results

ECT2 mRNA levels were positively correlated with WHO pathologic grading

The ECT2 expression level was higher in WHO grade IV ($n = 81$) then in grade II gliomas ($n = 7$; $P = 0.0126$) [Figure 1]. The ECT2 level was also higher in WHO grade IV glioma than in non-tumor controls ($n = 23$) ($P = 1.65 \times 10^{-8}$, P adjusted by Bonferroni method). These results confirmed that high-grade gliomas over-expressed ECT2.

High ECT2 expression correlated with poor survival in high-grade gliomas

The Kaplan-Meier survival analysis of 77 patients with WHO grade III and grade IV gliomas showed that patients with low ECT2 mRNA expression levels ($n = 67$) had better overall survival than those with high ECT2 mRNA expression levels ($n = 10$; $P < 0.0001$, by log-rank test; 95% CI: 0.02402–0.2565, Ratio 0.7850) [Figure 2]. The cut-off value was set at 645.95. The median survival interval in the high- and low-ECT2 expressions level was 46 weeks and 106 weeks, respectively.

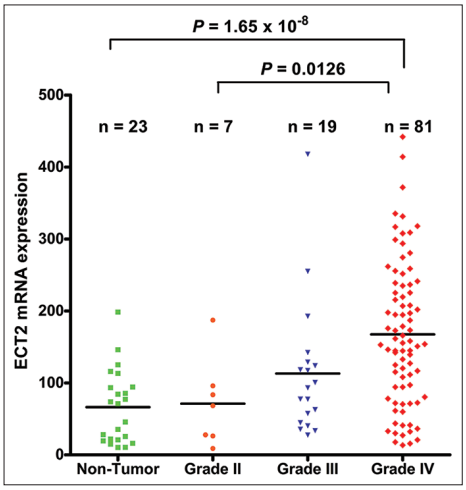

Figure 1: ECT2 mRNA expression in glioma and non-tumor control groups. The distributed plots demonstrated the ECT2 gene expression in low-grade glioma and non-tumor controls compared to those in high-grade gliomas (grades III and IV). Increased ECT2 mRNA levels positively correlated with WHO grades of gliomas. The adjust p value was calculated between each group

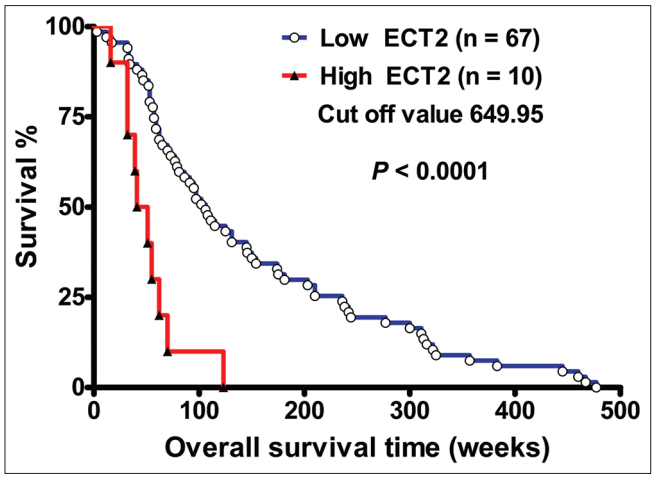

Figure 2: Overall survival in patients with high-grade gliomas. The Kaplan-Meier survival curve showed poor survival in those with high ECT2 (> 649.95; *n* = 10) compared to those with low ECT2 (< 649.95; *n* = 67) expression levels (median survival, 106 vs. 46 weeks, *P* < 0.0001, by log-rank test; HR: 0.07850, 95% CI: 0.02402–0.2565)

Discussion

The present study reveals that ECT2 expression was significantly higher in patients with high-grade gliomas than in those with low grade gliomas and in non-tumor controls. Furthermore, high ECT2 predicted poor survival as compared to low ECT2 expression. This is consistent with the findings by Sano *et al.*,[19] who also found that patients with higher ECT2 mRNA expression and protein levels in 54 glioma tissues had poorer outcomes. Similarly, Weeks *et al.*,[20] demonstrated that ECT2 expression rose in malignant primary astrocytomas. They further investigated if the molecular mechanisms of ECT2 regulated invasiveness and found that ECT2 was co-localized with RAC1 and CDC42 at the leading edge of migrating glioma cells. Interestingly, the sub-cellular distribution of ECT2 was abnormally localized to the cytoplasm in both primary human gliomas and glioma cell lines compared to normal cell, wherein ECT2 was localized within the nucleus. The inhibition of ECT2 expression resulted in the suppression of RAC1 and CDC42 activity. Their study revealed that elevated ECT2 level correlated with glioma invasiveness and progression, suggesting that ECT2 might be a biomarker for identifying patients with glioma progression.[20]

Moreover, the molecular mechanism of ECT2 also has been addressed in non-small cell lung cancer (NSCLC) such that ECT2 correlated with the PKCiota oncogene in NSCLC.[11] ECT2 translocated from the nucleus to the cytoplasm of NSCLC cells to bind with the PKCiota-Par6 oncogenic complex, which stimulated the Rac1 small GTPase. PKCiota phosphorylated ECT2 directly and RNAi-mediated knockdown PKCiota or Par6 resulted in a decrease in phospho-Thr-328 ECT2, indicating that in NSCLC cells, PKCiota regulated Thr-328 ECT2 phosphorylation. These studies provided evidence that ECT2 would bind to the oncogenic PKCiota-Par6 complex, thereby activating Rac1 motion and promoting tumor growth and invasion. Furthermore, RNA interference of ECT2 induced neurite outgrowth also revealed that ECT2 regulated the differentiation of NG108-15 neuroblastoma/glioma hybridoma.[24]

The limitation of this study included difficulty in collecting large amounts of human brain tumors, particularly non-tumor control or grade II low-grade gliomas, for examining mRNA expression or protein level. As an alternative, large-scale analysis of 280 sheets of data from GEO profiles[22,25] was used to confirm the role of ECT2 as a prognostic biomarker of poor outcome.

Conclusions

There is a positive correlation between ECT2 expression and different WHO pathologic grades of gliomas. In high-grade gliomas, ECT2 over-expression is correlated to poor survival, by multivariable analysis. Thus, ECT2 is related to pathologic grading of human gliomas and therefore may be a dependable biomarker for determining their overall survival or outcome

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