ENTPD1 (CD39) and NT5E (CD73) expression in human Glioblastoma: an in silico analysis

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Short Report

Keywords: Glioblastoma, in silico, ENTPD1, NT5E, CD39, CD73

Posted Date: May 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1636293/v1

Abstract

Glioblastoma is the most common primary brain tumor in adults. It has a poor prognosis and the available lines of treatment have barely improved patient survival over the past decade. ENTPD1 and NT5E are genes that encode the cell surface enzymes CD39 and CD73, respectively. Both act in a purinergic extracellular pathway, hydrolyzing ATP into adenosine, promoting tumor immunosuppression, neovascularization and invasiveness. Thus, these genes emerge as genetic targets for new treatments for glioblastoma. We performed an *in silico* analysis of 156 tumor samples in an unexplored public database to investigate the prognostic value and consequent therapeutic potential for these genes. The analyzes revealed a significant increase in transcription levels of the genes under study in glioblastoma samples versus non-tumor brain tissue samples. High expression of NT5E or ENTPD1 is independently related to a decrease in overall survival ($p = 5.4e-04$, $1.1e-05$). NT5E transcriptional levels are significantly higher in wild-type glioblastoma IDH patients compared to mutant glioblastoma IDH, while ENTPD1 levels show no significant differences, $p \leq 0.001$. Finally, patients with mutated glioblastoma IDH and wild-type IDH had significantly lower overall survival when transcriptional levels of target genes were increased ($p = 0.011$; $p = 3.2e-03$). These data indicate the need for further population studies that explore the value of ENTPD1 and NT5E not only as prognostic markers but also as potential therapeutic targets.

Introduction

Glioblastoma (GB) is the most common primary brain tumor in adults, with an incidence of 3.19:100.000 inhabitants, affecting mainly the elderly population [1]. The average survival time can vary from 3 months (if no treatment is received) to up to 16 months on average if the patient undergoes maximum safe resection, concurrent radio and chemotherapy [1,2]. Virtually all patients relapse after 10 months [2] and only about 17.5% of patients survive after the 2nd year of diagnosis [1]. GB is feared not only for its high mortality but also for the devastating impact on the quality of life associated with the natural history of the disease.

As a grade IV glioma, atypia, mitosis, necrosis, and vascular infiltration define its heterogeneous histology. However, grouping gliomas into molecular types has been used modernly and, according to Gravendeel *et al.*, 2009 [3], genotypes are more accurate predictors of survival than histology, assuming great importance in clinical decisions. The 2016 WHO classification for Central Nervous System tumors incorporated genetic markers [4] such as the isocitrate dehydrogenase (IDH) gene mutation, which is associated with younger age presentation and longer survival, in contrast to patients with wild-type IDH gene (wild type or WT) [5]. In addition, the MGMT gene mutation also predicts longer survival and a better response to adjuvant treatment. Other genes such as EGFR, TP53, ATRX, TERT have also been studied in gliomas, but they have less clinical relevance at this time [1].

CD73 is a cell surface enzyme encoded by the NT5E gene that participates in the purinergic pathway in synergy with CD39, encoded by the ENTPD1 gene, promoting the hydrolysis of extracellular ATP into adenosine (ADO) [6]. In the tumor microenvironment, ADO promotes tumor progression not only by

stimulating adhesion, proliferation, invasion and angiogenesis, but also by inducing immunosuppression of tumor-associated immune cells, mainly by stimulating its A2b receptor. This specific receptor is also associated with the glioma chemoresistance profile [7,8]. Both CD73 and CD39 are overexpressed in GB tumor tissue [7, 9, 10].

The prognostic and therapeutic potential of these genes has been explored in contemporary literature, however, it has not yet been incorporated into treatment [7, 11]. Translational experimental studies have shown that CD39 and CD73 silencing is associated with decreased tumor growth [12, 13]. A recent *in silico* analysis explored the relationship between CD73, GB and intratumoral NK cell infiltration. This study showed that the degree of expression of CD73 is inversely related to disease-free survival time. Patients with high expression of both CD73 and CD39 genes had a shorter survival than others. The survival time between patients with high expression of only one of these genes and patients with low expression of both genes was similar [10].

Based on this, we asked whether the degree of independent expression of NT5E/CD73 and ENTPD1/CD39 is associated with a shorter survival time in patients diagnosed with GB. To investigate the prognostic value and consequent potential therapeutic target of CD73 and CD39 in this disease, we conducted an *in silico* analysis of 156 representative tumor samples in unexplored public databases [3]. We then generated Kaplan-Meier curves for survival time in samples with high and low expression of these genes. Therefore, the study aims to evaluate the expression of CD73 and CD39 mRNA comparing tumor and non-tumor tissue from the samples and to correlate with the survival time of patients diagnosed with GB from a public dataset.

Methodology

The mRNA expression of target genes was normalized in all samples within 'R2: Genomic Analysis and Visualization Platform (http://r2.amc.nl) and presented in graph format as log2 transformed from signal intensity. The classification of subgroups and the number of patients in each subgroup was performed according to the availability of stratification in the database (French - https://hgserver1.amc.nl/cgi-bin/r2/main.cgi). The analysis separates the samples from the dataset into high and low gene expression. Each expression value was ordered ascendingly as a cutoff point to form two groups and test the p-value in a log rank test. The test will find the maximum significant expression cut-off point for the survival analysis. Therefore, we found the best possible Kaplan Meier curve by the Log rank test. All subgroups were compared using a Kruskal-Wallis test for significance and the False Discovery Rate method, followed by the post-hoc Welch t test performed using the R2 platform. For gene expression analyses, $p \leq 0.001$ values were considered statistically significant. The study was exploratory.

Overall survival (OS) was measured from date of initial diagnosis to death or date of last follow-up, using OS combined with gene expression data according to the availability of each database. Survival distribution was estimated according to the Kaplan-Meier method using a median cut-off and log-rank statistics; $p \leq 0.05$ was considered statistically significant.

An institutional ethical approval was not required for this study, and it was not pre-registered.

No sample calculation was performed, becouse all available data that could be used were used.

Results

In silico analysis of the dataset of samples from 156 GB patients revealed a significant increase in transcriptional levels of NT5E and ENTPD1 when compared to normal brain tissue or non-tumor brain tissue (control), $p \leq 0.001$.

Kaplan-Meier curve analysis showed that high NT5E or ENTPD1 expression in GB samples is significantly related to decreased overall survival (p = 5.4e-04, 1.1e-05). $p \leq 0.05$ was considered significant.

Transcriptional levels of target genes were also evaluated in samples from GB patients carrying the r132 IDH mutation (n = 33), compared to WT IDH (n = 95). It was found that transcriptional levels of NT5E are significantly higher in patients with GB IDH WT compared to the mutated GB IDH, while ENTPD1 levels do not show significant differences between samples.

In the analysis of OS associated with transcriptional levels of target genes NT5E and ENTPD1, both patients with GB IDH WT and patients with mutated IDH had a significantly lower OS when the transcriptional levels of these genes were increased.

Discussion

Enzymes of the isocitrate dehydrogenase (IDH) group catalyze the oxidative decarboxylation of isocitrate and, therefore, play important roles in cell homeostasis. The presence of the mutated IDH predicts a median survival of 31 months, whereas patients with IDH WT have a median survival of 15 months [4]. This difference was corroborated in the present study.

CD39 is an enzyme expressed on the cell surface that hydrolyzes extracellular ATP to AMP, which can later be converted to ADO by the action of CD73, thus working together in the adenosinergic cascade. Extracellular ATP is a pro-inflammatory metabolite that binds to P2X receptors on T cells and induces cytokine production. Therefore, its hydrolysis by CD39 compromises the functionality of effector T cells and favors the increase in regulatory T cells. This inappropriate activation of T cells results in dysfunctional $CD8^+$ T cell states, including anergy, exhaustion, and senescence [14].

CD73 is a regulator of glioma oncogenesis: on the one hand, its expression favors the growth, migration and invasion of GB cells by producing ADO; on the other hand, its “downregulation” reduces the viability of gliomas and enhances the effect of temozolomide [12]. High NT5E expression is a prognostic predictor of lower overall survival in patients with the GB mesenchymal subtype [10].

This *in silico* analysis corroborated data in the literature that NT5E and ENTPD1 are overexpressed in GB when compared to normal brain tissue [7, 9, 10]. Furthermore, it also found that a higher expression of

these genes in tumor tissue is associated with a lower overall survival when compared to the expression levels of these genes among patients with GB. This finding reinforces the importance of high expression of both genes as predictors of prognosis and points to the need for further studies that seek to analyze the correlation of expression of these genes with the prognosis of patients with GB.

We also found that patients with GB IDH WT have higher expression of NT5E but not ENTPD1 when compared to patients with the IDH r132 mutation. Furthermore, we found that high expression of both NT5E and ENTPD1 is related to decreased overall survival regardless of IDH status. These data were not previously described and may contribute to the understanding of the worst prognosis of these patients. This finding reinforces the importance of the expression of CD73 and CD39 in tumorigenesis and, therefore, as prognostic markers and potential targets for the development of new treatments.

Conclusion

GB is a disease with a poor prognosis and the available lines of treatment have barely improved patient survival in the last decade. The present study corroborates data already present in the literature and explores new genetic markers.

GB IDH WT patients have a worse prognosis than mutated GB IDH patients and express higher levels of CD73 but not CD39 in their tumor samples, demonstrating its potential as a marker. The degree of expression of CD73 and CD39 is associated with a shorter median survival time in patients diagnosed with GB, regardless of IDH status.

These data indicate the need to carry out more population studies that explore the value of CD73 and CD39 not only as prognostic markers but also as potential therapeutic targets.

Declarations

Acknowledgments

The authors would like to thank the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS; process numbers 19/2551-0001779-0 and 21/2551-0000078-3), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – process numbers 312187/2018-1; 400882/2019-1), Hospital de Clínicas de Porto Alegre (Número CAAE: 49967921500005327).

Conflict of interest

The authors declare no confict of interest or competing interests.

Ethical approval

This work does not involve animals or human subjects.

Consent for publication

All the authors provided their consent for the publication of this study.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript

Competing Interests

The authors have no relevant financial or non-financial interests to disclose

Author Contributions

Marco Antonio Stefani and Elizandra Braganhol contributed to the study conception and design. Material preparation, data collection and analysis were performed by Marco Antonio Stefani and Elizandra Braganhol. All authors contributed to writing this first draft of the manuscript. All authors read and approved the final manuscript.

Data Availability

The data can be obtained upon request to the corresponding author or in https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?open_page=login

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