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Increased Expression of ZEB1 in Glioma

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Abstract

Glioma is the most common primary brain tumor in adults with a high proportion of cases displaying malignant features. Based on WHO classification, glioma can be graded from I to IV according to their histological features and malignant properties, with grade IV, known as GBM, showing the highest level of malignancy. Meningioma, the tumor arising from the meninges, is a primary benign brain tumor in 80% of the cases. It is classified into grades I to III with grade III demonstrating malignant characteristics. However, the lower grade, benign tumors of meningioma are significantly more prevalent.

Radiation therapy, chemotherapy and surgery have long been considered as the first-line treatment strategies for brain tumor patients and yet, the scarcity of efficient treatment strategies encourages researchers to seek the major molecules that are implicated in tumor progression, metastasis and recurrence. Therefore, this study has been carried out to find some of the major molecules in tumor invasion and metastasis through a comparison of the majorly benign tumors of meningioma with the majorly malignant tumors of glioma in the hope of introducing molecular targets for more accurate diagnostic and targeted therapy purposes. One of these significant molecules is ZEB1 with its diverse roles in tumor progression, metastasis and invasion.

This study included 44 meningioma samples alongside 31 glioma samples. Expression of ZEB1, a major molecule implicated in a plethora of malignancy processes, has been investigated by real-time PCR technique and the significance of the results has been analyzed statistically. The results, in agreement with our hypothesis, demonstrated a significant up-regulation of ZEB1 in glioma samples compared to meningioma, suggesting this molecule as a plausible target for diagnostic purposes or targeted therapy in the future.

Introduction

Glioma and Meningioma are two common primary brain tumors worldwide. Glioma, as the most prevalent malignant primary brain tumor in adults, represents 81% of malignant brain tumors. It arises from glial cells and is categorized into four grades based on the World Health Organization (WHO) revised classification for brain tumors [1]. Glioblastoma Multiform, known as GBM, is the most malignant and most common type of glioma (grade IV) and comprises 45% of glioma cases with high invasion and migration ability. GBM incidence is 0.59-3.69 per 100,000 people [1]. Meningioma, which originates from the meninges, presents as a benign tumor of the brain in approximately 80% of cases. Additionally, it accounts for about one third of the central nervous system (CNS) tumors and is classified into three grades based on the WHO classification (grades I-III), with grade III meningioma tumors representing malignant features. The WHO classification for meningioma has prognostic markers but lacks aggressiveness and recurrence markers. However, a significant proportion (about 80 percent) of meningioma tumors occur as low-grade (grade I) meningioma, making benign meningioma more prevalent [2] with 20% displaying 5-year recurrence for completely removed tumors. Although surgery and radiation therapy are first-line treatments for glioma and meningioma, there is scarcity of reliable molecules for design of targeted therapies or as biomarkers for accurate diagnostic purposes, especially in regard to tumor aggressiveness [1-2]. Therefore, understanding the molecular mechanism of tumorigenesis in brain is necessary to find significant molecules for efficient diagnosis and targeted therapies.

Glial stem cells [3], cellular migration and invasion [4], Driver mutations [5], and more importantly, Epithelial-To-Mesenchymal Transition (EMT) play major roles in brain tumor aggressiveness and pathogenesis. Epithelial-to-Mesenchymal Transition is a process by which tumor cells lose their epithelial features and gain mesenchymal features, rendering them capabilities of relapse and metastasis. A plethora of signaling pathways such as Hh, Notch and TGF-B are known to be implicated in the EMT process [6]. Several transcription factors have been proven to contribute to EMT such as ZEB1 [7], TWIST1 [8] and SNAIL [9] ZEB1, or Zinc finger E-box homeobox-1, is a transcription factor which is implicated in tumor invasion ad metastasis through the EMT process. It is known to fulfill DNA repair functions in the face of ionizing radiation exposure. Additionally, it is believed to suppress the expression of E-cadherin, an epithelial biomarker, and induces the expression of mesenchymal phenotypes through suppressing its targets such as Crumb3, HUGL2, and PATJ which ultimately culminate in tumor metastasis. ZEB1 also exerts drug-resistance dependent or independent of EMT [7].

To help elucidate some molecules implicated in the aggressive behavior of malignant tumors and to further help clarify the roles of these biomarkers in identification of malignant tumors from benign tumors, the ZEB1 mRNA expression level was investigated in meningioma and glioma fresh samples. Another objective of this

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study is to investigate role of ZEB1 in patient prognosis and above all to come up with more specific and suitable molecular candidates to be used in the future as an aggressiveness biomarker for therapeutic or diagnostic purposes. Roles of the very same molecule in aggressiveness have been previously analyzed in basal cell carcinoma as the least aggressive/metastatic skin cancer [10]. The present research, as a pilot study, aimed to evaluate the mRNA expression level of ZEB1 (an EMT molecule with aggressiveness potential) in glioma samples, which have aggressive features compared to meningioma samples which have benign features for the most part. Finally, significant up-regulation of ZEB1 mRNA expression is seen in glioma samples compared to meningioma samples with p=0.017.

Material and Methods

Sample collection, RNA extraction, cDNA synthesis and Real-Time PCR forty-four meningioma samples and thirty-one glioma samples were collected from Shariati Hospital affiliated to Tehran University of Medical Sciences during 2014-2016, after having obtained the patient's informed written consent. The pathology reports for all the samples were observed for confirmation of the tumor type and definitive diagnosis of their grade. RNA was extracted with Tripure isolation reagent (Roche, Mannheim, Germany) based on the manufacturer's protocol. All RNA was treated with Recombinant DNase I (Takara Bio Inc, Shiga, Japan) according to the manufacturer's protocol to eliminate remaining DNA content based on the manufacturer's instructions. Then, RNA concentration was measured and also agarose gel electrophoresis was performed to confirm complete elimination of any remaining DNA. cDNAs were synthesized by the PrimeScript RT reagent (TakaraBio Inc, Shiga, Japan) according to the manufacturer's protocol. ZEB1 primer was designed by Primer-BLAST (NCBI) and efficiency was checked utilizing Gene Runner 6.5.51. The sequence of the designed ZEB1 forward primer was CATTTTTCCTGAGGCACCTG and the ZEB1 reverse primer was GGAACACCAGATGCATTTTCA. Quantitative real-time PCR was performed by Light Cycler® 96 System (Roche Life Science, Germany) using SYBR Premix Ex Tag TM (Takara Bio Inc, Shiga, Japan).Real-time PCR cycles will be given upon request. At the end of each Real-time PCR, a melting curve was performed to confirm the specificity of the amplicon peaks and absence of any primer dimer peaks. Expression data for each tissue sample was normalized by GAPDH gene expression data as the housekeeping gene.The GAPDH primer sequence was obtained from our previous study. All experiments were performed in duplicates.

Statistical Analysis

Data was presented with mean. The Q-Q plot and the Kolmogorov-Smirnov test were used to evaluate normal distribution. Analysis of variance (ANOVA), Chi-square and Fisher's exact test were done to compare groups. The ROC curve and area under the curve were used to evaluate the sensitivity and specificity of each mRNA. Statistical analysis was performed with SPSS (IBM Corp. Released2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBMCorp.).A p value less than 0.05 was deemed statistically significant for a confidence interval of 95%.

Results and Discussion

Glioma and meningioma fresh samples were obtained with mean age of 43 for glioma patients and 57 for meningioma patients. After sample preparation, quantitative Real-Time PCR was performed for evaluating ZEB1 mRNA expression. In this study, glioma samples, as the most common malignant primary brain tumor, and meningioma samples, as the benign type of brain tumor were analyzed to find the role of ZEB1 mRNA expression in the aggressiveness of the tumor samples. Comparing mean relative expression of ZEB1 in glioma samples versus meningioma samples, significant up-regulation of ZEB1 mRNA was demonstrated (p=0.017), as shown in Figure 1. Since ZEB1 has been considered to be implicated in the more aggressive behavior of malignant tumors, ZEB1 mRNA expression displayed marked, statistically significant increase in our glioma samples in comparison with meningioma, which suggests that there might be further molecular mechanisms regulating the aggressive feature of gliomas. Congruent with our hypothesis, a previous study reported correlation between ZEB1 expression in glioblastoma patients and poor prognosis [11].

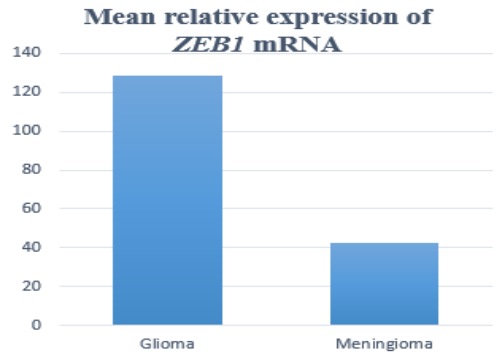

Figure 1: Mean relative Expression of *ZEB1* mRNA expression in glioma samples versus meningioma samples.

Our study reports up-regulation of ZEB1 in glioma samples compared to meningioma samples, and, so far, previous studies have also reported up-regulation of ZEB1 protein mostly in invasive types of glioma samples [11]. Dysregulation of ZEB1 mRNA expression level was expected in glioma samples compared to meningioma samples, however, this finding signals the need to further study whether if there are any other molecular pathways modulating ZEB1 expression and also whether if EMT molecules might have significant role in glioma invasion and aggressiveness. ZEB1 is one of the regulatory transcription factors which has an important role in EMT. As mentioned before, EMT is a complex process and is modulated by many regulatory molecules and a plethora of studies have shed light on its role in tumor migration, invasion and metastasis. Herein, to investigate the role of EMT in brain tumor aggressiveness and invasion, ZEB1 was selected because it has been previously analyzed and proven as a key regulator of EMT in other cancers including bladder cancer [12], pancreatic cancer [13], and breast cancer [14]. Therefore, up-regulation of ZEB1 mRNA expression in glioma samples compared to meningioma samples might provide new insight into EMT in brain tumor aggressiveness and invasion. However, due to a small sample size, our findings need further analysis with an extended sample size.

New molecules could be targeted for future approaches of treatment regimens and diagnosis since the existing conventional

types of brain tumor treatments, including surgery, chemotherapy and radiation therapy have low efficiency. Gliomas and, more importantly, high-grade gliomas (grade III and GBM) are infiltrative tumors capable of invading the surrounding brain parenchyma. This deems glioma complete surgical resection impossible and therefore very probable to recur and exhibit resistance to therapy. Therefore, this study was performed with the hope of finding new molecular targets for more efficient, specific, recently-emerging diagnosis, prognosis, and gene therapies and targeted therapies. Increasing hope is emerging for use of new targets in treatment of tumors in the upcoming years [15].

Conclusion

Meningioma is a well-known example of a benign brain tumor comprising approximately one-third of CNS tumors and absolutely zero metastasis and relapse capability, making it a great *in vivo* model to be compared to malignant tumors of the brain namely glioma accounting for a high proportion (81%) of brain tumor cases with intracranial invasion and aggressiveness. Thus, comparing glioma and meningioma samples could be an appropriate *in vivo* tumor model to uncover molecular mechanisms contributing to aggressiveness and invasion in brain tumors.

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