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Human leukocyte antigen-G overexpression predicts poor clinical outcomes in low-grade gliomas

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ABSTRACT

Overexpression of human leukocyte antigen-G (HLA-G), a non-classical major histocompatibility complex class-I molecule associated with immunosuppression, has been reported in various human malignancies. In the present study, we examined the role of HLA-G in gliomas. Clinical characteristics, mRNA expression microarrays and follow-up data pertaining to 293 patients with histologically confirmed gliomas were analyzed. The expression levels of HLA-G were compared between different grades of gliomas and correlated with progression-free survival (PFS) and overall survival (OS) to evaluate its prognostic value. We found that HLA-G was overexpressed in gliomas as compared to that in normal brain tissue samples (-1.288 ± 0.265). The highest expression levels were in glioblastomas (GBMs), anaplastic gliomas (AGs) and low-grade gliomas (LGGs), in that order (0.328 ± 0.778, 0.176 ± 0.881, -0.388 ± 0.686, respectively). Significant inter-group differences were observed between low-grade and high-grade glioma tissues ($p < 0.001$ and $p < 0.001$, *t*-test, AGs and GBMs, respectively). More astrocytoma patients exhibited increased HLA-G expression as compared to other LGG patients ($p = 0.004$, Chi-square test). Significant differences were observed with respect to PFS and OS ($p = 0.009$ and 0.032, log-rank test, for PFS and OS, respectively) between the high- and low-expression subgroups in patients with LGGs. On Cox regression analysis, overexpression of HLA-G appeared to be an independent predictor of clinical outcomes ($p = 0.007$ and 0.026, for PFS and OS, respectively). Our results suggest that HLA-G expression may serve as a potential biomarker for predicting aggressive tumor grades of gliomas and for histological subtype of LGGs. Elevated HLA-G expression could serve as an independent predictor of poor clinical outcomes in patients with low-grade gliomas.

1. Introduction

Glioma, the most common and most lethal primary tumor of the central nervous system, accounts for more than 70% of all brain tumors (DeAngelis, 2001; Ohgaki and Kleihues, 2005). Gliomas are typically very difficult to resect completely, and often recur after surgery. Thus, they represent a challenge in neuro-oncology (DeAngelis, 2001; Ohgaki and Kleihues, 2005). For the most frequent and aggressive type of glioma, glioblastoma multiforme (GBM), the median duration of survival after standard treatment with maximal surgical resection and subsequent radio-chemotherapy is only 14.6 months, and the two-year survival rate is below 30% (Stupp et al., 2009; Stupp et al., 2005). Although recent studies have contributed to the knowledge of gliomas, yet the molecular basis of this cancer needs to be fully elucidated.

Evasion of immune surveillance and suppression of the immune system are hallmarks of cancer. Schreiber first proposed and improved the concept of cancer immunoediting, which results from the interaction between innate and adaptive immune cells in the tumor microenvironment, local inflammation and the immune response of the host (Dunn et al., 2002; Vesely et al., 2011; Vesely and Schreiber, 2013). The concept of cancer immunoediting refers to the complex interaction between tumor cells and the immune system, and to the dual role of immune system in the development of cancer, i.e., both tumor suppression and tumor progression (Manjili, 2011; Mittal et al., 2014; Schreiber et al., 2011). Up to 20% of all clinical tumors are related to an underlying chronic inflammation, wherein the immune cells stimulate tumor growth and progression (Grivennikov et al., 2010). The immunosuppressive property of human glioma is well-characterized (Wiendl et al., 2002). However, the underlying mechanisms which allow it to evade the host immune system are not completely understood.

The major histocompatibility complex (MHC) refers to a group of coding genes for the cell surface proteins which modulate the immune response. The human MHC is also called human leukocyte antigen (HLA) complex. HLA gene is located at the 6p21.31 region on the short

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arm of human chromosome 6. It is a 4000 kb gene complex which consists of hundreds of gene groups. It is known for its maximum allelic polymorphisms and is closely related to the regulation of human immune system (Reipert, 2014; Rouas-Freiss et al., 2003). Based on the gene sequence, it is generally divided into three groups: HLA-I (e.g., HLA-A, HLA-B, HLA-C, HLA-E, and HLA-G), HLA-II (e.g., HLA-DR, HLA-DP, and HLA-DQ) and HLA-III (Nakajima et al., 2010). Altered expression of HLA has been documented in a variety of inflammatory or malignant conditions. The association of HLA expression with certain human diseases has also been demonstrated. For instance, in the early 1970's a strong correlation between HLA-B27 and ankylosing spondylitis was detected in more than 90% patients (Schlosstein et al., 1973). Similar association has also been demonstrated between HLA and human cancers (e.g. cervical cancer and breast cancer) (Jia et al., 2015; Kochan et al., 2013).

The association between glioma and HLA gene expression has been reported. A positive correlation between HLA-E expression and survival status was found in patients with glioblastoma (Kren et al., 2011). In a recent study, overexpression of HLA-DR was shown to be associated with poor prognosis in glioma patients (Diao et al., 2015). In our previous study, we identified an association between HLA-G expression and radiologic morphology in low-grade gliomas (LGGs) (Wang et al., 2015). To the best of our knowledge, no study has yet investigated the role HLA-G plays in different grades of gliomas. We assessed the mRNA expression level of HLA-G in glioma patients and explored its potential correlation with different clinicopathological parameters.

2. Methods

2.1. Patients and tissue samples

Gene microarray data pertaining to 293 patients with glioma was retrospectively analyzed. Surgically resected brain tissues obtained from 5 trauma patients served as negative controls. Histological diagnosis of gliomas was independently performed by two experienced neuropathologists according to the 2007 World Health Organization classification (Louis et al., 2007). The tumor tissues were obtained from patients after surgical resection between June 2007 and September 2013 in the Department of Neurosurgery, Beijing Tiantan Hospital. None of the patients died of other diseases or unexpected events during evaluation. Clinical information of all patients was obtained from the institutional database. Follow-up data was available for all patients. The mean duration of follow-up was 31.5 ± 21.0 months (range 0.7–82.9 months; median, 27.0 months). The study was approved by the Ethics Committee of Beijing, Tiantan Hospital. Written informed consents were obtained from all patients. The tissue samples were flash frozen in liquid nitrogen immediately after resection and stored at −80 °C until further processing. In order to reduce the influence of normal cell contamination, the percentage of tumor cells in each sample was assessed by hematoxylin and eosin (H&E) staining, and only samples with >80% tumor cell content were selected for further analysis.

2.2. RNA extraction and whole genome profiling

RNA extraction and whole genome profiling were performed as described elsewhere (Wang et al., 2015). Total RNA from the frozen samples were extracted using a MirVana miRNA Isolation kit (Thermo Fisher Scientific, Waltham, USA). Subsequently, NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA) was used to measure the concentration and quality of the RNA.

Microarray analysis was performed on all 298 samples using the Agilent Whole Human Genome Array (Agilent, California, USA). The integrity of the total RNA was checked with an Agilent 2100 Bioanalyzer (Agilent, California, USA). Both cDNA and biotinylated cRNA were synthesized and hybridized onto the array. Data acquisition was performed using the Agilent G2565BA Microarray Scanner System and Agilent Feature Extraction Software (version 9.1). The probe intensities were normalized with GeneSpring GX 11.0.

2.3. Statistical analysis

Statistical analyses were performed using SPSS software 16.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant. Mean expression levels of HLA-G were compared between patients with different grades of gliomas using Student's *t*-test. Based on their HLA-G expression levels, the glioma patients of each tumor grade were further subdivided into two subgroups (cut off at 50% of the entire group) for further analysis. Univariate analysis was performed using Chi-square test for dichotomous clinical variables. Kaplan–Meier analysis (log-rank test) was used to evaluate predictive value of HLA-G expression level for main outcomes such as progression-free survival (PFS) and overall survival (OS) in patients with different grades of gliomas. Patients who had expired without evidence of any other disease were regarded as censored events for OS, and tumor recurrences as diagnosed by MR images were regarded as censored events for PFS. Cox's proportional hazards model was used to determine the independent association of prognostic variables with PFS and OS.

3. Results

3.1. Patient characteristics

Clinical and mRNA expression microarray data for all 293 patients were obtained. Among these, 117 patients were staged as grade II (Low-grade glioma), 50 as grade III (Anaplastic glioma, AG) and 126 were staged as grade IV (Glioblastoma multiforme, GBM). At the time of diagnosis, the median age of subjects was 38 years (range 18–62 yrs). Baseline patient characteristics are summarized in Table 1.

3.2. Expression of HLA-G in gliomas of different grades

HLA-G expression in all different grades (II–IV) of glioma tissues was significantly higher as compared to that in the normal brain tissue (0.016 ± 0.829 versus −1.288 ± 0.265, $p < 0.001$, *t*-test). Expression levels of HLA-G in 5 normal brain tissues are summarized in Supplementary Table 1. HLA-G gene was highly overexpressed in gliomas, especially in GBMs (0.328 ± 0.778), then in the AGs (0.176 ± 0.881) and LGGs (−0.388 ± 0.686). Significant differences were observed between low-grade and high-grade glioma (AGs and GBMs) tissues ($p < 0.001$ and $p < 0.001$, *t*-test, AGs and GBMs, respectively, Fig. 1) while no significant differences were observed between AGs and GBMs ($p = 0.264$, *t*-test, Fig. 1).

3.3. Association of HLA-G with clinicopathological features

Chi-square test was used to examine any potential correlation between HLA-G expression and clinical characteristics (Table 2). A significant difference in HLA-G expression was found between LGG patients with astrocytomas and those with oligodendrocytic tumors ($p = 0.004$, Chi-square test). Greater number of patients with astrocytomas exhibited high expression levels of HLA-G, which suggested that in

Table 1
Baseline characteristics of glioma patients (N = 293).

Variables	Grade II (N = 117)	Grade III (N = 50)	Grade IV (N = 126)
Median age (range)	37 (18–62)	41.5 (18–66)	47.5 (12–70)
Sex (male)	54	28	79
Pathology (astrocytoma)	66	14	–
KPS > 80	71	22	34

Grade II: Low-grade glioma; Grade III: Anaplastic glioma; Grade IV: Glioblastoma multiforme; KPS, Karnofsky performance score.