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Original Article

Gene expression profiling by mRNA array reveals different pattern in Chinese glioblastoma patients between Uygur and Han populations

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Abstract: *Objective:* To identify differentially expressed genes in Chinese glioblastoma patients of Uygur and Han populations, and investigate their potential clinical value for pathogenesis determination and progress prediction. *Methods:* Gene expression profiling was obtained from three patients of each Uygur and Han nationalities, respectively, by mRNA expression array. Data were processed by the GenomeStudio software and language R of the Lumi package, followed by GO (Gene Ontology) term and KEGG pathway annotation analysis by the Web Gestalt software. *Results:* The comparative analysis of genome-scale gene expression in glioblastomas revealed 1,475 differentially expressed genes, with 669 and 807 genes up-regulated and down-regulated, respectively. These included the STRC gene, which has two transcripts, one up-regulated and one down-regulated. GO term analysis suggested that 1,175 out of 1,475 key genes were involved in small GTPase mediated signal transduction, Ras protein signal transduction, bioprocess of neuronal response regulation, and central nervous system myelination. The KEGG pathway enrichment analysis showed that the differentially expressed genes were covered by 28 signaling pathways associated with tumorigenesis, including metabolic pathways, tumor suppressor pathways, MAP kinase signaling pathways, TGF-β signaling pathway, neurotrophin signaling pathways, and mTOR signaling pathway. *Conclusion:* The comparative study of gene expression profiling in glioblastomas between Uygur and Han nationalities revealed differentially expressed genes, whose functions and expression localization were analyzed by GO term analysis and KEGG pathway enrichment analysis. Different pathogenesis mechanisms were proposed for glioblastomas in Chinese patients of Uygur and Han nationalities from a molecular biology perspective.

Keywords: Glioblastomas, han population, uygur population, gene expression profile, biological function

Introduction

Primary glioblastoma is the most common malignancy of the central nervous system. It is also known as Grade IV Astrocytoma by WHO, and usually affects adults, especially those at middle and older age. Glioblastomas commonly occur in the cerebral hemisphere and most of them are primary tumors without obvious low-grade precancerous lesion period [1]. Glioblastoma is of high malignancy with short survival period and unfavorable prognosis once diagnosed. In recent years, glioblastoma has been increasingly diagnosed in younger individuals, with a mortality rate close to 100% [2]. Xinjiang autonomous region is a multi-ethnic area of China, with the populations of Uygur and Han nationalities at nearly equal proportions (hereinafter referred to as Uygur and Han populations). While the Han race belongs to the Mongolians, Uygurs belong to caucasoid. A total of 212 glioblastoma patients admitted to our hospital underwent surgery, including 135 men and 77 women, with 71 Uygur and 141 Han individuals. Uygur patients included 41 males and 30 females averaging 44.4 years; Han patients included 94 men and 47 women of 50.1 years in average. Uygur patients were younger and had severer disease (stage III or IV by WHO) compared with Han patients, with 53.5% at

Table 1. Clinical data of glioblastoma patients

Serial number	No.	Nationality	Gender	Age	Tumor location	Maximum diameter/cm	WHO classification
1	3258	Uygur	Woman	29	Left frontal lobe	4	IV
2	6114C	Uygur	Man	51	Left frontal lobe	2	IV
3	6501	Uygur	Man	44	Right frontal lobe	4	IV
4	2968C	Han	Woman	50	Left frontal lobe	2	IV
5	1746C	Han	Woman	56	Right top lobe	1	IV
6	1414	Han	Woman	39	Left frontal lobe	4	IV

Table 2. Quality control of total RNA samples from six glioblastoma patients

Serial number	Sample No.	Concentration ng/ml	A260/A280	Total amount (mg)	Electrophoresis
1	3258	1611	2.02	39.78	Qualified
2	6114C	739.8	1.93	19.37	Qualified
3	6501	2703.2	2.01	67.08	Qualified
4	2968C	597.8	1.92	15.54	Qualified
5	1746C	279.3	2.03	6.94	Qualified
6	1414	1588.7	2.02	39.22	Qualified

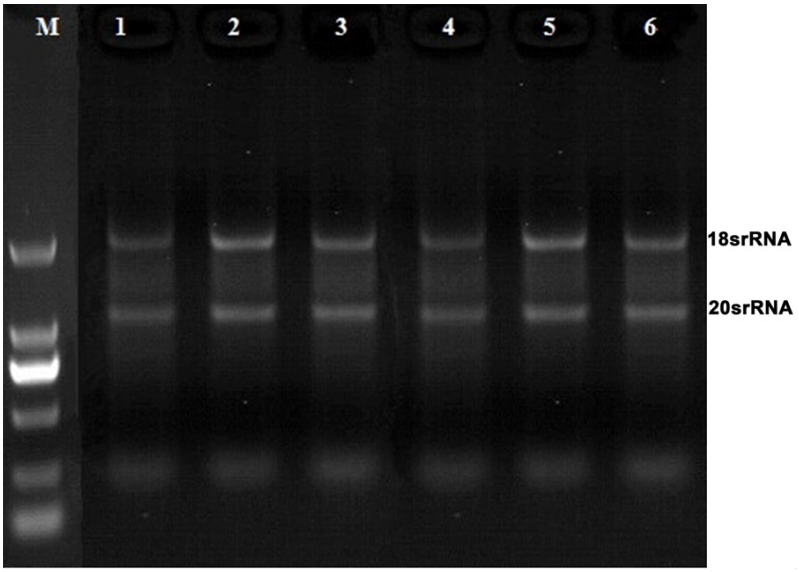

Figure 1. Agarose electrophoresis of total RNA from six glioblastomas patients. M, DNA markers; lane 1 to 6, samples from individual patients.

advanced stages; those with relatively less advanced grades were transformed to advanced tumor grades after recurrence. Only 47.5% Han patients were at advanced stages. In general, glioblastomas is of high malignancy with very high risk of recurrence after surgical removal.

The difference between Uygur and Han glioblastoma patients in clinical manifestations suggested that differences at the gene level may account for the pathogenesis. In the current study, mRNA expression array was utilized to detect differentially expressed genes between Uygur and Han glioblastoma patients, to investigate the potential mechanisms of pathogenesis.

Materials and methods

Patients

Fresh tissue samples were obtained from six patients randomly selected from those who underwent glioblastoma removal surgery in the affiliated tumor hospital of Xinjiang Medical University between January 2010 and December 2013; all patients were diagnosed by histology after operation. These 6 patients have similar living environment and diet habit. There were three patients of each Uygur and Han, respectively, including four men and two women with ages ranging between 29 and 56 years (median, 44 years). Histology classified the glioblastomas from all six patients as grade IV, and tissues were stored at -80°C. The clinical data of each patient was complete, including age, gender, nationality, pathological

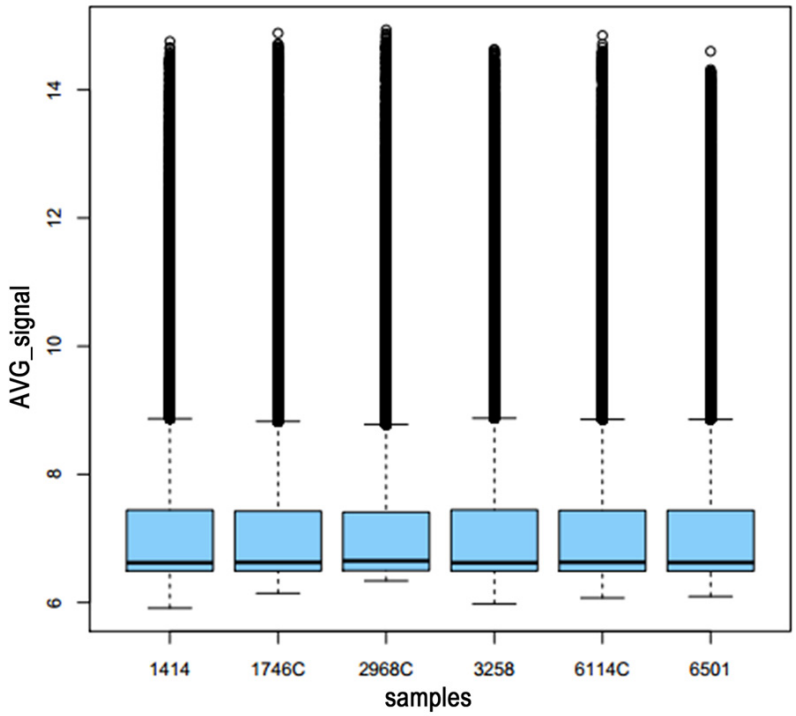

Figure 2. Line-box map of equilibrium analysis among patients

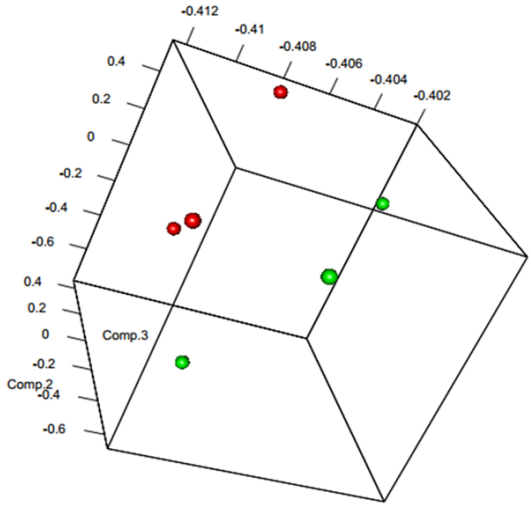

Figure 3. PCA map of equilibrium analysis among patients.

diagnosis, imaging analysis, and follow-up data post-surgery. None of the six patients was receiving radiotherapy or chemotherapy. The study was reviewed by the ethics committee preoperatively and informed consent was signed by all patients for specimen sampling and storage (**Table 1**).

Total RNA isolation and measurement

Total RNA was prepared by Trizol reagent (Life Technologies, USA) and the RNeasy kit (Qiagen, Germany) according to manufacturers' guidelines. The RNA concentrations quality control was performed by 1% agarose electrophoresis and NanoDrop Spectrophotometer 2000 (NanoDrop, USA).

mRNA expression array detection and quality control

Total RNA was in vitro reverse transcribed to cDNA, and mixed with hybridization reagents. The mixture was incubated at room temperature, and washed once at high temperature, followed by ethanol washing and three sequential washings at room temperature. Finally, the Illumina HT-12mRNA microarray (Illumina, USA) was dried and scanned, to acquire data and corresponding images.

There were six different internal controls in mRNA expression array, and a total of 887 probes for quality control in each sample. The quality controls included hybridization controls, negative controls, biotin and high stringency, gene intensity, low stringency, and controls on labeling and background. All tissue samples passed quality control assessments such as principal component analysis (PCA) and sample grouping balanced contrast analysis, indicating the experimental process was normal with qualified probes and the generated data reliable.

Results

Quality and quantity assessment of total RNA

Total RNA concentrations were greater than 100 ng/μl, with total amounts above 2 μg. The purity was determined by NanoDrop 2000, and A260/A280 ratios were greater than 1.90

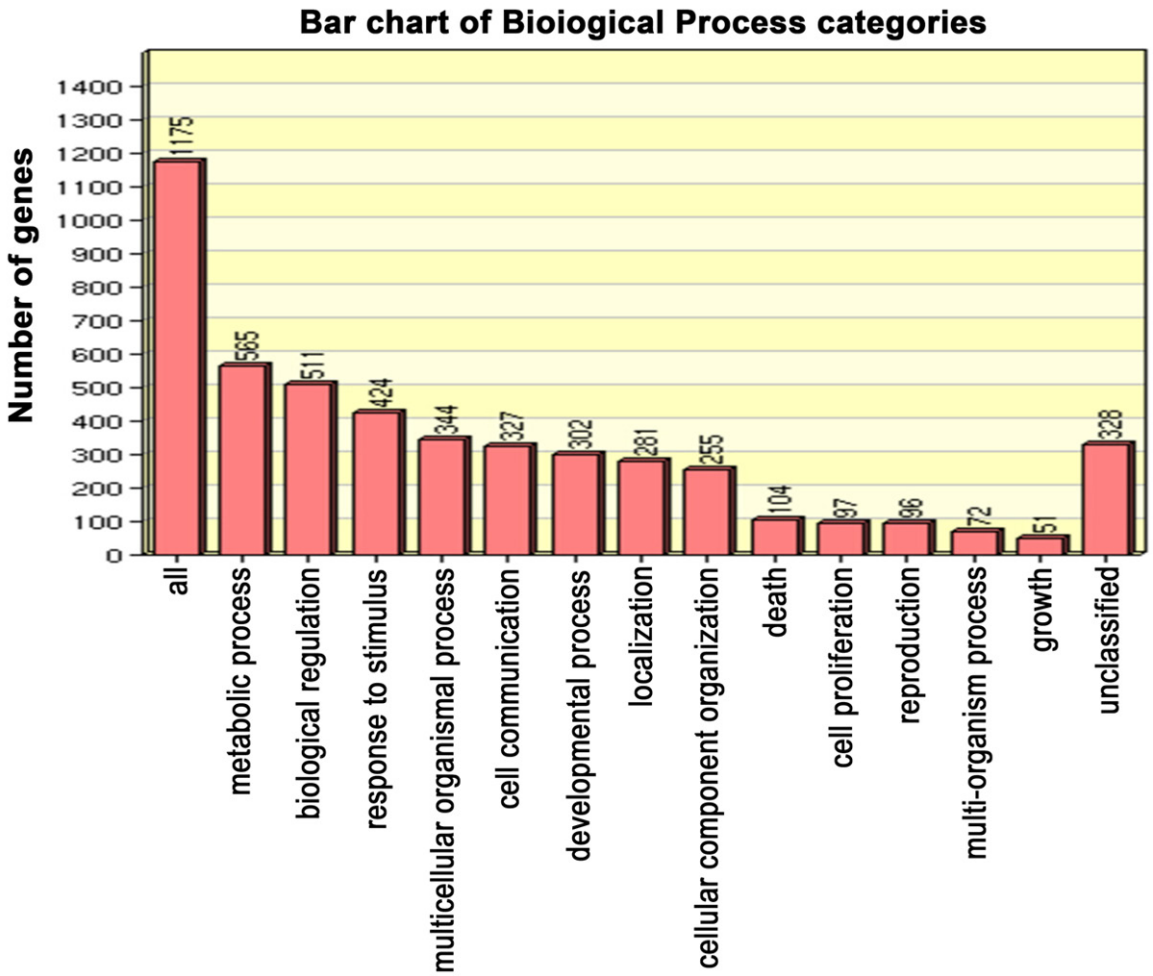

Figure 4. Biological involvement of differentially expressed genes.

(**Table 2**). 1% agarose electrophoresis showed two clear bands corresponding to 18 srRNA and 28 srRNA (**Figure 1**), indicating the integrity and high purity of the total RNA isolated, with no degradation; these samples were therefore appropriate for subsequent mRNA expression microarray detection.

Equilibrium analysis of microarray data

There were three samples from each Uygur and Han glioblastoma patients included in the current study. The equilibrium analysis indicated a symmetrical distribution of the acquired data, with similar median and central tendency; therefore, the data among patients were comparable. As shown in box-line (**Figure 2**), the expression of all probes in each sample was analyzed by principal component analysis, and samples of each group were distributed in different regions of the three-dimensional space; meanwhile, data from one patient were relatively concentrated in the same spatial location. Red dots representing data from Uygur patients were concentrated in one region; whereas, the green dots representing data from Han individuals were in another region. The expression profiles of the two groups were very different, though there was relatively high internal consistency. The data from same group were relatively concentrated in the same spatial region, indicating an accurate representativeness and biological repeatability, as shown in (**Figure 3**).

Differentially expressed genes and bioinformatics analysis

Scan images of the hybridized mRNA arrays were processed by the Illumina GenomeStudio

Table 3. Biological processes involving the differentially expressed genes

GO-ID	Biological process	Items	Term enrichment	*P* value
GO: 0007264	Small GTPase mediated signal transduction	61	1.68	4.75e-05
GO: 0007265	Ras protein signal transduction	42	1.90	4.71e-05
GO: 0001508	Action potential	22	2.70	1.86e-05
GO: 0019228	neuronal action potential	21	3.57	2.63e-07
GO: 0007272	Ensheathment of neurons	18	3.62	1.55e-06
GO: 0008366	Axon ensheathment	18	3.62	1.55e-06
GO: 0042552	Myelination	17	3.54	4.05e-06
GO: 0032291	Axon ensheathment in central nervous system	7	8.34	6.43e-06
GO: 0022010	Central nervous system myelination	7	8.34	6.43e-06

Table 4. Distribution of differentially expressed genes in KEGG pathways

Name of pathway	*P* value	Differentially expressed genes
Metabolic pathway	5.84e-10	MGAT3, COX15, UGT8, GALNT6, SI, DPAGT1, CKMT2, GGPS1, B3GNT5, BCAT2, ALOX15, ITPK1, CYP4F11, NDUFV3, ACOX2, EN01, ZNRD1, MMAB, GLDC, MCEE, GCNT2, IDH3A, HADHA, ACSM5,CPOX,AC01,PPAP2C,ST3GAL5,MAN2A2,LSS,ALDOA, MLYCD, PIK3C2B, ACSBG2, BCKDHA, GAMT, DHCR24, ADO, HSD17B10, PLD2, ETNK2, INPP5B, CHKA, ADH5, GART, FPGS, PAICS, QDPR, SYNJ2, AGL, POLR1E, POLR3F, AGPAT4, RDH10, LDHA, MAN1C1, CNDP1, ST6GALNAC3, GLCE, GK2, LIPT1, POLR1D, ACLY, HK2, HMBS, PLD1, HAO2, SPTLC3
Tumorigenesis pathways	4.16e-06	TRAF6, TGFA, WNT7A, RASSF1, RALGDS, FIGF, CHUK, PTK2, VEGFA, DAPK2, PIAS3, BAD, JUN, RB1, CRK, RALB, NCOA4, MYC, WNT5B, CKS1B, PLD1, STAT3, HRAS, FZD1, TRAF6, ATF4, MAP4K4, MAPKAPK5, RASGRP3, CH
MAP kinase pathways	5.39e-05	UK, IL1B, PPM1B, MAP2K3, HSPA1B, JUN, RPS6KA2, CRK, MYC, ARRB1, DUSP10, RRAS2, HRAS, HSPA2
TGF-β signaling pathway	9.65e-05	BMP2, ZFYVE16, ID, THBS4, MYC, E2F5, INHBE, FST
Neurotrophic factor signaling pathways	0.0007	JUN, BAD, TRAF6, SORT1, ATF4, RPS6KA2, CRK, ZNF274, SH2B1, MAP3K5, HRAS
mTOR signaling pathway	0.0028	FIGF, RICTOR, RPS6KA2, VEGFA, PRKAA1, ULK2

software to obtain the corrected dataset. Then, standardization and differentially expressed gene analysis were carried out by language R of the Lumi package ($P < 0.05$). A comparative analysis between Uygur and Han patients for genome-scale glioblastoma gene expression revealed 1,475 differentially expressed genes, with up-regulation of 669 genes and down-regulation of 807 genes. These included the STRC gene, which has two transcripts, with one up-regulated and one down-regulated. All the differentially expressed genes, known in public databases, were analyzed by the Web Gestalt software for GO (Gene Ontology) term and KEGG (Kytoencyclopedia of genes and genomics) pathway annotation ($P < 0.05$).

GO includes terms such as 'Biological Process', 'Cellular Component', and 'Molecular Function'. All 1,475 genes underwent GO term classification, and gene term enrichment analysis by statistical tests, and it showed a significant clustering of these differentially expressed genes. 1,175 genes out of 1,475 were mainly involved in metabolic process, biological regulation, response to stimulus, multicellular organismal process, cell communication, and cell proliferation (**Figure 4** and **Table 3**).

KEGG pathway analysis is a collection of manually curated databases dealing with genome and systematic biological function analysis. The differentially expressed genes underwent KEGG classification, followed by enrichment significance calculation; the less the *P* value is, the more significant the enrichment is considered to be. KEGG analysis indicated that differentially expressed genes were involved in mul-

tiple tumorigenesis pathways, and highly enriched in 28 pathways including metabolic process, tumorigenesis pathways, MAP kinase pathways, TGF-β signaling pathway, neurotrophic factor signaling pathway, and mTOR signaling pathway (**Table 4**).

Discussion

Glioma is the most common primary brain tumor, with more than half being the most malignant glioblastoma. The median survival rate is not high even when the patients had undergone the most proactive therapies. The preoperational diagnosis is mainly based on imaging examination and clinical manifestations. Postoperative histological examination is the major tool for glioblastoma classification according to WHO. Compelling studies have indicated that the molecular mechanisms, therapy strategies, and prognosis are all very different in various classifications of glioblastomas [3, 4].

In recent years, glioblastoma studies have focused on the expression of TP53, MGMT, and VEGF in Xinjiang [5, 6], and genome-scale analyses are scarce. The overt difference between Uygur and Han patients regarding pathogenesis and clinical features indicated that variability at the gene level might account for disease mechanisms.

Inevitably, the study of disease mechanisms at the gene level is needed for glioma characterization. Similar to other malignant tumors, occurrence and development of glioblastoma result from a series of molecular alterations. Since Schena *et al* published the first paper on gene expression array in 1995, microarrays have been widely used for multiple malignancies, including ovary tumors, breast cancer, prostate cancer, and cervical cancer [7], as well as glioma. However, differentially expressed genes in Uygur and Han patients have not been reported.

The study of differentially expressed genes in glioblastoma patients of Uygur and Han has focused on common molecular biomarkers such as MGMT, TP53, and IDH [8], which are mentioned in diagnosis guidance. It was reported that the occurrence of more than 50% of malignant tumors is associated with TP53 gene mutations, which had an incidence rate around 25 to 37% in glioblastomas. In addition, the expression of the TP53 protein in low-grade gliomas indicated unfavorable outcome; however, TP53 mutation was not a valuable prognosis indicator for glioblastoma [9]. O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme, repairing DNA alkylation damage caused by nitrosourea chemotherapy. Interestingly, glioblastomas with low MGMT expression were shown to respond to treatment with alkylating drugs; these tumors are otherwise resistant to alkylating drugs [10]. There are three isoforms that constitute the isocitrate dehydrogenase (IDH) family: IDH1, IDH2, and IDH3. IDH mutation was shown to be positively associated with TP53 mutation, loss of heterozygosity on chromosome lp19q, and MGMT promoter methylation status. Multiple studies have indicated that IDH mutation and MGMT promoter methylation are typical biomarkers of glioblastomas [11-13].

Besides, the differentially expressed genes identified in the current study included many well-known molecules such as BMP2, ADH5, ERCC1, DAPK2, GAB2, HLA-DP/DR, interleukin families, MMP16, MAPKAPK5, NOTCH2, PIK families, PCNA, RASSF1, RB1, S100A1, TF, VEGFA, and TP73L.

The differentially expressed genes between Uygur and Han patients covered multiple biological processes, including metabolic process, biological regulation, response to stimulus, and multicellular organic process; there are also involved many signaling pathways such as small GTPase signaling transduction, Ras protein signal transduction, bioprocess of neuronal response regulation, and central nervous system myelination, and closely associated with multiple tumorigenesis pathways, including metabolic process, tumorigenesis pathways, MAP kinase pathways, TGF-β signaling pathway, neurotrophic factor signaling pathways, and mTOR signaling pathway [14-18]. Studies assessing single candidate genes only provide limited information, whereas, whole pathway analysis could elucidate the synergetic effect of a group of genes. In the current study, KEGG pathway analysis revealed 28 signaling pathways enriched, and multiple genes involved in metabolic and tumorigenesis pathways indicated their potential pathogenesis mechanism.

There were common differentially expressed genes between Uygur and Han patients, but also nationality specific differentially expressed genes, indicating some common underlying mechanisms for the pathogenesis of glioblastomas, despite the overt genetic difference between both nationalities.

The analysis of differential gene expression between Uygur and Han patients provides an in-depth understanding of the molecular biological pathogenesis and signaling pathways of glioblastomas. The distinctive expression profiles in patients from both nationalities broaden our knowledge on glioblastoma pathogenesis in different genetic backgrounds. It also provides certain theoretical basis for the future specific gene treatment in patients of Han and Uygur glioma.

Disclosure of conflict of interest

None.

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