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Original Article

Identification of meningioma recurrence gene expression signature by DNA microarray experiments

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Abstract: Meningioma recurrence after complete removal remains one of the most relevant problems of meningioma treatment. DNA microarray technologies allow the screening of several thousands genes simultaneously. This gene expression profiling approach has been successfully applied in many researches associated with tumor classification. We analyzed genome wide expression profiles of 68 meningioma samples. The differential gene expression analysis was conducted to identify meningioma recurrence gene expression signature. The gene set enrichment analysis and Cox proportion hazards methods were used to characterize the gene signature. A total of 99 genes (65 up and 34 down) were identified as significantly regulated in recurrent meningioma tumors. These genes mainly enriched in the biological process of cell cycle. Among them, seven genes were significantly associated with meningioma patients' overall survival. The cell cycle genes may play a vital role in the meningioma progression. However, further research is required to validate our findings and discover novel treatment and prognosis potentialities.

Keywords: Meningioma recurrence, DNA microarray, Cox proportion hazards models

Introduction

Meningioma recurrence after complete removal remains one of the most relevant problems of meningioma treatment [1, 2]. In fact, the recurrence rate has been estimated to be 9-15% [3, 4] within 10 years for benign tumors and 38% [5] at 5 years for atypical tumors. Many radiological [6, 7] and surgical [8] features have been proposed to be associated with this aggressive behavior, there are little agreements among these factors. The mechanisms of meningioma recurrence are still not clear.

DNA microarray technologies allow the screening of several thousands genes simultaneously. This gene expression profiling approach has been successfully applied in many researches associated with tumor classification [9-11]. In DNA microarray analysis, the oncologists prefer to focus on the expression signature of small subsets of genes that distinguish the outcomes, such as tumor recurrence, before conducting in-depth study.

Our aim in this study was to examine molecular profiles of recurrent and non-recurrent meningioma tumors to determine meningioma recurrence associated gene signatures. To meet this end, the DNA microarray technology was used to measure genome wide expression profiles in 68 meningioma tumors. With use of these data, gene expression comparison analysis was performed and the gene set enrichment analysis was conducted to characterize the gene signature. Of importance, the identified gene signature was also analyzed for the relationship with overall survival. The results presented here are of great help in understanding the mechanisms of meningioma recurrences.

Methods

Patients and tissue samples

In the present study, a total of 68 patients (25 males and 43 females with a median age 64; range 32 to 89 years) were considered. 10 out of these meningioma patients progressed recurrence. As summarized in **Table 1**, accord-

Table 1. Clinical characteristics of patients with meningioma tumors

Demographics	Training Dataset, *n* = 68
Median age, years (range)	64 (32-89)
Gender	
Male	25
Female	43
Recurrence	
Yes	10
No	58
Grade	
I	43
II	19
III	6

ing to WHO criteria, 43 patients (63.24%) were diagnosed as grade I. 19 and 6 patients were diagnosed as grade II and grade III respectively.

DNA microarray experiment

As reported previously [12], RNA was extracted from 20-50 mg tumor pieces using QiagenTM (Valencia, CA) RNA easy mini kits per manufacturer's protocols. The extracted total RNA was assessed for integrity using the 2100 Bioanalyzer by Agilent TechnologiesTM (Santa Clara, CA). 1 g of total RNA was used for single-round biotinylated probe synthesis using the Affymetrix Array Station device made by Caliper Life Sciences (Hopkinton, MA) by manufacturer's protocols. Labeled and sheared cRNA was manually applied to Affymetrix Human Genome U133 Plus 2.0 Arrays (Santa Clara, CA). All microarrays were scanned using the Affymetrix GeneChip 3000 scanner. All images were manually examined to confirm that none had surface defects and exhibited proper grid alignment.

DNA microarray data procession

The DNA microarray raw fluorescence intensity datasets were retrieved from NCBI GEO database with accession number GSE16581. The R software (http://www.r-project.org/) with packages through Bioconductor (http://bioconductor.org/) was used to pre-process the DNA microarray datasets. The gcrma [13] algorithm was implemented to perform background correction and data normalization, during which optical noise and non-specific binding were considered to adjust the background intensities in Affymetrix array data. The resulted log 2 gene expression values were then processed with the Bioconductor limma [14] function to calculate differential gene expression between recurrent and non-recurrent meningioma samples. The limma function introduces a moderated t statistic and calculates a false discovery rate (FDR) among groups [14]. The meningioma recurrence gene expression signature was defined as genes showed differential expression in meningioma recurrence samples ($|LogFC| \geq 1$ and LogOdds >4.6). A log-odds value greater than 4.6 indicates 99% probabilities that gene is differentially expressed between the conditions being compared.

Characterization of gene expression signature

After the gene expression signature was identified, the DAVID (The Database for Annotation, Visualization and Integrated Discovery) tools [15, 16] were used to analyze the Gene Ontology [17] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. To investigate the prognostic prediction potential of the gene signature, the Cox proportional hazards method was implemented to perform univariate analysis.

Results

A total of 68 meningioma tumors were measured using Affymetrix Human Genome U133 Plus 2.0 Arrays (Santa Clara, CA). The DNA microarray raw fluorescence intensity datasets were retrieved from NCBI GEO database and a bioinformatics pipeline was implemented for data pre-procession. Then the differential gene expression was examined to determine meningioma recurrence associated gene signature.

As shown in **Figure 1**, a total of 65 genes were significantly up regulated in recurrent meningioma tumors, while 34 genes were identified as down regulated. The online bioinformatics tool, DAVID, was used to look for enrichment of genes annotated in Gene Ontology entities or KEGG pathways. Top ten biological processes significantly enriched among up-regulated genes in recurrent meningioma tumors included M phase (GO:0000279), cell cycle phase (GO:0022403), cell division (GO:0051301),

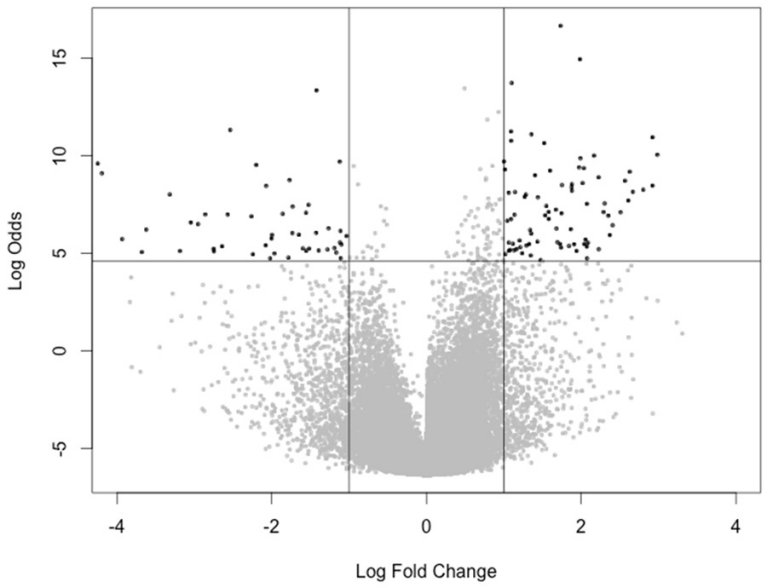

Figure 1. Volcano plot of log-fold changes versus log-odds of differential expression. The x-axis indicates the log2 value of fold-change between the two conditions. The Log Odds (or B value) on the y-axis is the odds (or probability) that the gene is differentially expressed. The black dots located in the upper left and right square are genes that have a log-odds score of 4.6 or more, have a fold change greater than 2-fold, and are identified as significantly differentially expressed in meningioma recurrence samples.

Table 2. List of genes significantly correlated with meningioma prognosis

Gene symbol	Regulated in recurrent meningioma	Logrank *P*-value	Hazard Ratio (95% Confident Internal)
KLHDC1	Down	7.05E-03	0.34 (0.15-0.78)
TSPAN7	Down	4.48E-02	0.72 (0.52-1.00)
ZNF516	Down	1.10E-02	0.42 (0.21-0.85)
NOV	Down	3.12E-03	0.66 (0.49-0.89)
CLU	Down	1.84E-03	0.46 (0.28-0.78)
WHSC1	Up	4.68E-02	2.45 (0.99-6.04)
TRIM59	Up	1.04E-03	2.05 (1.26-3.33)

cell cycle (GO:0007049), mitotic cell cycle (GO:0000278), cell cycle process (GO:0022402), mitosis (GO:0007067), nuclear division (GO:0000280), M phase of mitotic cell cycle (GO:0000087) and organelle fission (GO:0048285). The down-regulated genes were significantly enriched in cell projection morphogenesis (GO:0048858), cell part morphogenesis (GO:0032990), rhythmic process (GO:0048511), cell morphogenesis (GO:0000902), cell projection organization (GO:0030030), cellular component morphogenesis (GO:0032989), neuron differentiation (GO:0030182) and positive regulation of cell-substrate adhesion (GO:0010811). The gene set enrichment analysis revealed that the up-regulated genes were significantly enriched in three KEGG pathways: Cell cycle (hsa04110, P = 8.10E-07), Oocyte meiosis (hsa04114, P = 1.34E-03) and p53 signaling pathway (hsa04115, P = 3.72E-02).

Among regulated genes in recurrent meningioma tumors, seven genes including KLHDC1, TSPAN7, ZNF516, NOV, CLU, WHSC1 and TRIM59 were significantly associated with meningioma patients' overall survival with Logrank test *P*-value < 0.05 (**Table 2**). From **Table 2**, it is evident that the up-regulated genes were associated with poor prognosis, while the down-regulated genes were associated with good prognosis. Since these genes could successfully distinguish poorer prognosis group from meningioma patients, they may serve as prognostic biomarkers or drug targets in the clinical treatment of meningioma tumors.

Discussion

In this study, molecular profiling of 68 meningioma tumors was performed to determine meningioma recurrence gene expression signature. With the use of DNA microarray data, a total of 99 genes were identified significantly regulated in recurrent meningioma tumors. Gene Ontology and KEGG pathway based gene set enrichment analysis revealed that the meningioma recurrence gene expression signature mainly enriched in the biological process of cell cycle. The progression of cell cycle is accomplished through a reproducible sequence of events, including DNA replication and mitosis. Mitosis (M phase) is separated temporally by G1 and G2 phases. The cell cycle control is considered to be central processes in the biol-

ogy of cancer [18]. Many cell cycle biomarkers have already been identified to be associated with tumor progressions [19-22]. Interestingly, among the meningioma recurrence gene expression signature, seven genes were significantly associated with prognosis, but they were not annotated in cell cycle processes. We cannot conclude that the cell cycle is not associated with meningioma prognosis, but the seven prognostic biomarkers are highlighted from the gene expression signature. Since these genes are involved in meningioma progression and clinical outcome, further research could be conducted to investigate their biological mechanisms and drug target potentiality.

Conclusion

In summary, we conducted differential gene expression analysis to identify meningioma recurrence gene expression signature. A total of 99 genes were identified as significantly regulated in recurrent meningioma tumors and enriched in the biological process of cell cycle. However, further research is required to validate our findings and discover novel treatment and prognosis potentialities.

Disclosure of conflict of interest

None.

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