Expression analysis of genes and pathways associated with liver metastases of the uveal melanoma

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Abstract

Background: Uveal melanoma is an aggressive cancer which has a high percentage metastasizing to the liver, with a worse prognosis. Identification of patients at high risk of metastases may provide information for early detection of metastases and treatment.

Methods: Expression profiling of ocular tumor tissues from 46 liver metastatic uveal melanoma samples and 45 non-metastatic uveal melanoma samples were got from GEO database. Bioinformatic analyses such as the Gene Oncology and Kyoto Encyclopedia of Genes and Genomes were used to identify genes and pathways specifically associated with liver metastases of the uveal melanoma.

Results: A total of 1138 probes were differentially expressed in two group samples. All differential gene interactions in the Signal-Net were analyzed. Of them, 768 probes were up-regulated and 370 down-regulated. They mainly participated in 125 GO terms and 16 pathways. Of the genes differentially expressed between two group cancers, HTR2B, CHL1, the ZNF family, YWHAZ and FYN were the most significantly altered.

Conclusions: Bioinformatics may help excavate and analyze large amounts of data in microarrays by means of rigorous experimental planning, scientific statistical analysis and collection of complete data about liver metastases of uveal melanoma patients. In the present study, a novel differential gene expression pattern was constructed and advanced study will provide new targets for diagnosis and mechanism of uveal melanoma liver metastases.

Keywords: Uveal melanoma, Liver metastases, Gene expression, GO analysis, Pathway analysis

Background

Uveal melanoma is the most common primary intracel- lular tumor in adults with an estimated 5-year survival rate of 50%-70% [1]. About 50% of patients develop metastases within a median of 36 months, mostly to the liver, with a median survival of 6 months after metasta- ses [2]. It seems important to identify high-risk patients at the time of the initial diagnosis for early detection and treatment of metastatic disease or for the administration of adjuvant therapy. Several clinical and histopatho- logical features have been correlated with survival, in- cluding patient age (>60), anterior location of the tumor, tumor cell histology, largest diameter of the tumor, mi- totic activity, and chromosome 3 monosomy. The most frequent chromosomal imbalances in uveal melanoma are loss of chromosome 3 and gains of 8q and 6p [3].

Despite the improvements in diagnosis and the devel- opment of more effective local therapies for primary tumors, the rate of metastatic death remains unchanged. Unfortunately, once uveal melanoma has spread to dis- tant organs, the disease is largely resistant to currently available therapies [4]. Nowadays, many new prognostic factors such as Cytological Features, Standard Karyotyping, Fluorescence in situ Hybridization, Centromeric Probes, Single Nucleotide Polymorphism and Gene Expression Profiling were investigated. Following the technique ad- vance and lower expense of gene expression microarray, it has become a useful tool for studying the development and progression of tumors owing to its high throughout,
genes which may affect metastases of uveal melanoma could be found.

Several gene expression profiling studies have identified two molecular classes strongly associated with metastatic risk [5-7]. Hepatocyte growth factor/scatter factor (HGF) [8-10], Insulin-like growth factor [11] and Stem cell factor [12-14] receptors have been involved in metastatic progression of uveal melanoma. In addition, the chemokine receptor CXCR4 was recently related to liver homing of human uveal melanoma metastatic cells [15,16]. However, little is known about the core genes and their potential mechanisms in liver metastases. The present study made use of bioinformatics method to analyze the data obtained from two public available datasets in combination with the clinical data about metastases of uveal melanoma patients in attempt to investigate the liver metastases-related genes. The different gene ontology and pathways would indicate the most important mechanisms and candidate genes in the process of liver metastases, and helpful in working out more specific and individualized target treatment regimens according to genetic characteristics of individual patients.

**Methods**

**Tissue samples and clinical data**

Tissue samples were obtained from two datasets in total 92 uveal melanomas after enucleation surgery upon approval of the institutional bioethics board. The patients in the two datasets were composed of 63 and 29 samples, respectively [17,18]. All the samples were performed on Affymetrix Human Genome U133 Plus 2.0 Array. Both of two data series were accessible at NCBI GEO database, accession GSE22138 and GSE27831. Clinical, pathological, and molecular features of the tumors are presented in Table 1.

**Significant differential gene analysis**

All 92 tumors in two datasets were pooled and reanalyzed on Affymetrix Expression Console Software (Version 1.1). MAS5 was used to normalize the original data. One sample was removed after the normalized data filtered with Pearson's Correlation (Additional file 1: Figure S1), and the remaining 91 samples were renormalized. Genes were standardized and interpreted functionally before comparison. Using t-test, SAM or RVM mode and the tumors with no metastases as the control group, the P value and the fold change were calculated for each differentially expressed gene. With a threshold of P value < 0.05 or FDR value < 0.05 and fold change > =1.5, related genes were picked out and the venn diagram was showed in Additional file 2: Figure S2. Based on our sample size and previous study [19], t-test result was chose for further analyze. Unsupervised hierarchical clustering was performed with Cluster using Pearson’s correlation distance metric and average linkage followed by visualization in Treeview [20].

**Gene ontology (GO) analysis**

Based on Gene Ontology Database (http://www.geneontology.org/), the significant level of GOs of the liver metastases-related differentially expressed genes was analyzed by two-side Fisher’s exact test and χ² test using DAVID (http://david.abcc.ncifcrf.gov/home.jsp) analysis [21]. The differential expression genes were analyzed independently according to up- and down-regulation of these genes. We computed P-values for all the differential expression genes in all GO categories, and the threshold of significance was defined as P-value < 0.05.

**Table 1 Clinicopathologic characteristics of uveal melanoma patients**

| Characteristic                  | Non-metastatic patients (n = 45) | Liver metastatic patients (n = 46) |
|--------------------------------|----------------------------------|-----------------------------------|
| Mean age, years                | 60.96 ± 14.4                     | 63.17 ± 10.21                     |
| Mean primary tumor thickness (mm) | 10.13 ± 2.97                     | 11.45 ± 2.76                      |
| Mean tumor largest diameter (mm) | 14.64 ± 4.14                     | 15.08 ± 4.01                      |
| Gender, No. of patients        |                                  |                                   |
| Male                           | 27                               | 29                                |
| Female                         | 18                               | 17                                |
| Location of tumor              |                                  |                                   |
| Anterior                       | 3                                | 4                                 |
| On equator                     | 29                               | 27                                |
| Posterior                      | 6                                | 13                                |
| All                            | 0                                | 1                                 |
| NA                             | 7                                | 1                                 |
| Histopathologic cell type      |                                  |                                   |
| Epithelioid                    | 9                                | 18                                |
| Spindle                        | 8                                | 18                                |
| Mixed                          | 16                               | 9                                 |
| NA                             | 12                               | 1                                 |
| Extrakreral extension          |                                  |                                   |
| No                             | 31                               | 31                                |
| Yes                            | 10                               | 9                                 |
| NA                             | 4                                | 6                                 |
| Chr 3                          |                                  |                                   |
| Monosomy                       | 17                               | 31                                |
| Disomy                         | 24                               | 6                                 |
| Partial monosomy               | 2                                | 3                                 |
| NA                             | 2                                | 6                                 |

Clinicopathologic characteristics of uveal melanoma patients got from the published article and each GSM information. NA, not available.
Pathway analysis

Based on KEGG (http://www.genome.jp/kegg/) database, the significant level of pathways of the liver metastases-related differentially expressed genes was analyzed by Pathway-Express [22,23]. Significant differences from the expected were calculated with a two-sided binomial distribution. The numbers of genes corresponding to each pathway category among the differentially expressed genes was tallied and compared with the number of genes expected for each pathway category based on their representation on the Affymetrix Human Genome U133 plus 2.0 array. All signaling pathways were analyzed for the significance level, using gamma P < 0.05 as the threshold.

Signal-net analysis

Using java that allows users to build and analyze molecular networks, network maps were constructed. For instance, if there is confirmative evidence that two genes interact with each other, an interaction edge is assigned between the two genes. The considered evidence is the source of the interaction database from KEGG. Networks are stored and presented as graphs, where nodes are mainly genes (protein, compound, etc.) and edges represent relation types between the nodes, e.g. activation or phosphorylation. The graph nature of Networks raised our interest to investigate them with powerful tools implemented in R.

Data analysis

Numerical data were presented as means and standard deviation (± SD). Differences between means were analyzed using Student's t test. All statistical analyses were performed using SPSS11.0 software (Chicago, IL).

Results

Clinical characteristics of the two group samples

Comparing with the non-metastases uveal melanoma samples, the liver metastases group has no differences

Table 2 Most obviously dysregulated genes sorted by P value in liver metastatic uveal melanoma compare to non-metastatic tumors

| Gene symbol | P value          | Mean of intensities: no metastasis | Mean of intensities: metastasis | Fold change |
|-------------|-----------------|------------------------------------|----------------------------------|-------------|
| NEURL1B     | 1.67 × 10⁻⁹     | 51.47                              | 88.42                            | 1.72        |
| EXT1        | 2.82 × 10⁻⁹     | 203.15                             | 362.35                           | 1.78        |
| DERL1       | 4.89 × 10⁻⁹     | 178.31                             | 412.54                           | 2.31        |
| GALNTL4     | 1.22 × 10⁻⁸     | 57.44                              | 128.49                           | 2.24        |
| M1R         | 1.41 × 10⁻⁹     | 60.08                              | 98.74                            | 1.64        |
| DDX39       | 3.31 × 10⁻⁹     | 347.31                             | 556.46                           | 1.60        |
| HTR2B       | 3.49 × 10⁻⁸     | 41.04                              | 574.49                           | 14.00       |
| RAB2A       | 5.29 × 10⁻⁸     | 363.13                             | 741.99                           | 2.04        |
| C10orf26    | 6.68 × 10⁻⁸     | 376.40                             | 569.30                           | 1.51        |
| GJC1        | 7.39 × 10⁻⁸     | 25.95                              | 75.05                            | 2.89        |
| CHL1        | 8.99 × 10⁻⁸     | 80.84                              | 17.12                            | 0.21        |
| ZNF33B      | 2.17 × 10⁻⁸     | 41.91                              | 21.24                            | 0.51        |
| OVS02       | 2.44 × 10⁻⁸     | 857.96                             | 185.87                           | 0.22        |
| EIF1B       | 3.35 × 10⁻⁷     | 1008.84                            | 567.87                           | 0.56        |
| PHLDA1      | 5.22 × 10⁻⁷     | 370.24                             | 134.93                           | 0.36        |
| PLSCR4      | 5.34 × 10⁻⁷     | 114.83                             | 53.41                            | 0.47        |
| MEGF10      | 8.38 × 10⁻⁷     | 165.39                             | 26.05                            | 0.16        |
| ZNF415      | 1.19 × 10⁻⁶     | 37.03                              | 13.91                            | 0.38        |
| ZNF667      | 1.25 × 10⁻⁶     | 34.08                              | 16.02                            | 0.47        |
| MEGF10      | 1.66 × 10⁻⁶     | 22.24                              | 10.21                            | 0.46        |

Most obviously dysregulated genes sorted by p value in liver metastatic uveal melanoma (n = 45) vs. non-metastatic tumors (n = 46).

Table 3 Most obviously dysregulated genes with fold change absolute value > 3 in liver Metastatic Uveal Melanoma compare to non-metastatic tumors

| Gene symbol | P value          | Mean of intensities: no metastasis | Mean of intensities: metastasis | Fold change |
|-------------|-----------------|------------------------------------|----------------------------------|-------------|
| HTR2B       | 3.49 × 10⁻⁸     | 5.36                               | 9.17                            | 14.00       |
| PPM1K       | 2.96 × 10⁻⁶     | 7.67                               | 9.39                            | 3.30        |
| SXX4        | 1.03 × 10⁻⁴     | 5.65                               | 7.28                            | 3.09        |
| PTGER4      | 3.01 × 10⁻³     | 6.75                               | 8.36                            | 3.06        |
| MEGF10      | 8.38 × 10⁻⁷     | 7.37                               | 4.70                            | 0.16        |
| SYNPR       | 1.25 × 10⁻⁵     | 9.15                               | 6.83                            | 0.20        |
| CHL1        | 8.99 × 10⁻⁸     | 6.34                               | 4.10                            | 0.21        |
| OVS02       | 2.44 × 10⁻⁷     | 9.74                               | 7.54                            | 0.22        |
| LOC100128252| 5.70 × 10⁻⁶     | 7.54                               | 5.55                            | 0.25        |
| MMPED2      | 5.58 × 10⁻⁶     | 6.28                               | 4.40                            | 0.27        |
| CNTN3       | 1.37 × 10⁻⁵     | 5.40                               | 3.60                            | 0.29        |
| PCDH20      | 2.94 × 10⁻³     | 5.82                               | 4.07                            | 0.30        |

Most obviously dysregulated genes with fold change absolute value > 3 in liver metastatic uveal melanoma (n = 45) vs. non-metastatic tumors (n = 46).
| GO item                                                                 | Fold enrichment | P value       |
|------------------------------------------------------------------------|-----------------|---------------|
| Germ cell programmed cell death                                        | 32.29           | 2.80 × 10⁻³   |
| Developmental programmed cell death                                    | 19.37           | 8.95 × 10⁻³   |
| Protein retention in ER lumen                                           | 13.84           | 1.80 × 10⁻²   |
| Germ cell migration                                                     | 13.45           | 3.65 × 10⁻⁴   |
| Melanocyte differentiation                                              | 9.22            | 8.27 × 10⁻³   |
| Mitotic cell cycle spindle assembly checkpoint                          | 8.81            | 4.36 × 10⁻²   |
| Negative regulation of mitotic metaphase/ anaphase transition           | 8.81            | 4.36 × 10⁻²   |
| Pigmentation during development                                         | 8.69            | 1.14 × 10⁻⁴   |
| Pigment cell differentiation                                            | 8.61            | 1.01 × 10⁻²   |
| Antigen processing and presentation of peptide antigen via MHC class I | 7.60            | 1.44 × 10⁻²   |
| Positive regulation of calcium ion transport into cytosol              | 6.46            | 2.26 × 10⁻²   |
| Regulation of calcium ion transport into cytosol                        | 5.57            | 1.16 × 10⁻²   |
| T cell proliferation                                                    | 4.78            | 4.95 × 10⁻²   |
| Regulation of transforming growth factor beta receptor signaling pathway| 4.14            | 3.15 × 10⁻²   |
| Positive regulation of homeostatic process                             | 4.04            | 3.42 × 10⁻²   |
| Response to vitamin A                                                   | 3.84            | 4.00 × 10⁻²   |
| Pigmentation                                                            | 3.83            | 9.50 × 10⁻³   |
| Mitotic cell cycle checkpoint                                           | 3.75            | 4.30 × 10⁻²   |
| Regulation of calcium ion transport into cytosol                        | 3.74            | 5.31 × 10⁻³   |
| Extracellular matrix organization                                       | 3.73            | 3.63 × 10⁻⁴   |
| Alcohol biosynthetic process                                            | 3.67            | 4.62 × 10⁻²   |
| Regulation of metal ion transport                                       | 3.59            | 3.49 × 10⁻³   |
| Leukocyte mediated immunity                                             | 3.38            | 5.04 × 10⁻³   |
| Cellular response to insulin stimulus                                   | 3.32            | 1.83 × 10⁻²   |
| Regulation of ion transport                                             | 3.26            | 3.51 × 10⁻³   |
| Lymphocyte mediated immunity                                            | 3.23            | 2.09 × 10⁻²   |
| Response to organic cyclic substance                                    | 3.20            | 1.30 × 10⁻³   |
| Response to toxin                                                       | 3.18            | 3.98 × 10⁻²   |
| Double-strand break repair                                              | 3.12            | 4.23 × 10⁻²   |
| Sphingolipid metabolic process                                          | 3.01            | 2.82 × 10⁻²   |
| Response to molecule of bacterial origin                                | 3.00            | 1.70 × 10⁻²   |
| Adaptive immune response                                                | 2.94            | 3.16 × 10⁻²   |
| Response to lipopolysaccharide                                          | 2.94            | 3.16 × 10⁻²   |
| Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains | 2.94            | 3.16 × 10⁻²   |
| Cellular response to hormone stimulus                                   | 2.91            | 2.77 × 10⁻³   |
| Response to insulin stimulus                                            | 2.91            | 1.22 × 10⁻²   |
| Membrane lipid metabolic process                                        | 2.79            | 3.91 × 10⁻²   |
| Activation of MAPK activity                                             | 2.76            | 4.11 × 10⁻²   |
on patients’ age, gender or tumor diameter. The tumor thickness value was much larger in the liver metastases samples than in the non-metastases group. Though the tumor location, extrascleral extension, tumor cell type and chromosome 3 data were incomplete, the first two appeared no differences while the mixed subtype tumor and chromosome 3 monosomy showed positive correlation with liver metastases of uveal melanoma (Table 1).

Liver metastases-related differential expression genes
Using liver metastases as the demarcation, genes of non-metastases and liver metastases groups were compared and 1138 statistically significant differential expression probes were obtained. Of them, 768 probes were up-regulated in the non-metastases samples and 370 were down-regulated. Hierarchical clustering showed systematic variations in the expression of genes between the two groups (Figure 1). The results demonstrated these differential probes could clearly separate the two groups from the whole samples and have good consistency in the group. Different genes with the most obvious p value and fold change between the liver metastases and non-metastases group were listed (Tables 2 and 3).

Significant GOs
GOs of the differential expression genes were statistically analyzed. It was found that the differential expression genes obtained from the Microarray mainly participated in 125 significant GOs. According to the enrichment list, the up-regulated differential expression genes mainly participated in 89 GOs including germ cell programmed cell death, developmental programmed cell death, germ cell migration, melanocyte differentiation (Table 4), and the down-regulated differential expression genes mainly participated in 36 GOs including embryonic skeletal system development, regulation of Rho protein signal transduction, regulation of cell morphogenesis (Table 5).

Significant Pathways
The pathways of liver metastatic uveal melanoma samples were analyzed according to the functions and interactions of the differential genes. By using Pathway-Express which contains both the up- and down- regulated differential genes in its analysis and the threshold of significance

Table 4 Different gene significant upregulated GO (Continued)

| GO item                                      | Fold enrichment | p value       |
|----------------------------------------------|-----------------|---------------|
| Phosphorylation                              | 1.57            | 5.41 × 10^3   |
| Oxidation reduction                         | 1.57            | 1.51 × 10^2   |
| Phosphate metabolic process                 | 1.56            | 2.49 × 10^3   |
| Phosphorus metabolic process                | 1.56            | 2.49 × 10^3   |
| Cell cycle process                          | 1.54            | 2.79 × 10^2   |
| Protein amino acid phosphorylation          | 1.50            | 2.49 × 10^2   |
| Immune response                             | 1.50            | 2.35 × 10^2   |
| Regulation of cell proliferation            | 1.44            | 3.02 × 10^2   |
| Regulation of programmed cell death         | 1.43            | 2.88 × 10^2   |
| Regulation of cell death                    | 1.43            | 3.01 × 10^2   |
| Regulation of apoptosis                     | 1.41            | 3.91 × 10^2   |

Table 5 Different gene significant downregulated GO

| GO item                                      | Fold enrichment | p value       |
|----------------------------------------------|-----------------|---------------|
| Calcium-dependent cell-cell adhesion         | 9.49            | 3.90 × 10^2   |
| Response to acid                            | 9.09            | 4.22 × 10^2   |
| Embryonic skeletal system development        | 5.67            | 4.04 × 10^3   |
| Response to vitamin                         | 5.51            | 1.26 × 10^3   |
| Regulation of cell shape                    | 5.39            | 3.75 × 10^2   |
| Regulation of Rho protein signal transduction | 5.14         | 2.36 × 10^3   |
| Pigmentation                                | 4.93            | 4.68 × 10^2   |
| Cartilage development                       | 4.91            | 1.85 × 10^2   |
| Skeletal system morphogenesis                | 3.90            | 1.88 × 10^2   |
| Regulation of cell morphogenesis            | 3.89            | 9.22 × 10^3   |
| Translational elongation                    | 3.60            | 4.97 × 10^2   |
| Muscle tissue development                   | 3.49            | 2.85 × 10^2   |
| Homophilic cell adhesion                    | 3.33            | 3.40 × 10^2   |
| Regulation of neuron differentiation        | 3.28            | 3.59 × 10^2   |
| Embryonic organ morphogenesis                | 3.28            | 3.59 × 10^2   |
| Regulation of cell development              | 3.19            | 7.23 × 10^3   |
| Regulation of Ras protein signal transduction | 3.12        | 8.25 × 10^3   |
| Muscle organ development                    | 3.10            | 8.54 × 10^3   |
| Regulation of neurogenesis                  | 3.07            | 2.66 × 10^2   |
| Response to drug                            | 3.03            | 9.75 × 10^3   |
| Embryonic organ development                 | 2.96            | 3.09 × 10^3   |
| Regulation of small GTPase mediated signal transduction | 2.89 | 7.88 × 10^3 |
| Skeletal system development                 | 2.74            | 4.38 × 10^3   |
| Heart development                           | 2.71            | 2.85 × 10^2   |
| Regulation of nervous system development    | 2.65            | 4.85 × 10^2   |
| Cell-cell adhesion                          | 2.64            | 1.37 × 10^2   |
| Negative regulation of cell proliferation   | 2.62            | 4.07 × 10^3   |
| Cell adhesion                               | 2.60            | 2.99 × 10^3   |
| Biological adhesion                         | 2.59            | 3.06 × 10^4   |
| Actin cytoskeleton organization             | 2.57            | 3.59 × 10^2   |
| Actin filament-based process                 | 2.41            | 4.79 × 10^2   |
| Chordate embryonic development              | 2.20            | 3.87 × 10^2   |
| Embryonic development ending in birth or egg hatching | 2.18 | 4.03 × 10^2 |
| Cytoskeleton organization                   | 2.17            | 1.68 × 10^2   |
| Regulation of cell proliferation            | 2.03            | 2.54 × 10^3   |
| Response to organic substance               | 1.71            | 3.85 × 10^2   |
defined on the basis of gamma P-value < 0.05, 16 significant pathways were found. Many signaling pathways had been verified to relate to cell migration and invasion, including Phosphatidylinositol signaling system, Gap junction, Adherens junction (Figure 2).

**Signal-network**

According to the literature and experimental records in the databases, a diagram of the gene interaction network was drawn up based on the genes differentially expressed between liver metastases and non-metastases uveal melanoma (Figure 3). The total number of genes in the network was 297, and the particular relationships between them were listed (Additional file 3). In the network, cycle nodes represent genes, and edges between two nodes represent interactions between genes, which were quantified by degree. Degrees within the network which describe the number of single gene that regulates other genes represent the size of the cycle node. The higher the degree, the more central the gene occurs within the network. There were many more genes upregulated involved in the Signal-Net analysis, of which YWHAZ, PRKDC, and ESR1 were the three main overexpressed genes, while the three main underexpressed genes were FYN, TIAM1 and GNAI1.

**Discussion**

The present study followed up microarray-based 91 uveal melanoma patients, of whom patients were not significantly different in age, gender and tumor diameter. More than 1100 probes were differentially dysregulated in liver metastases uveal melanoma in this study. Based on sorting the different genes by p value and fold change, numbers of genes with the largest difference individually were carried out. HTR2B is a type of serotonin receptor with both the extremely significant p value and fold change, numbers of genes with the largest difference in-...
expression genes participate in, based on which we can have a comprehensive understanding about interactions of genes, functions that they participate in and relations between up- and down-stream, and obtain genes involved in these significant pathways. Appearance of pathways about cell junction, melanogenesis and calcium signaling pathway confirm their concordance with GO terms and their critical role in liver metastases. Numerous studies had proved PI3K/Akt signaling pathway which belongs to phosphatidylinositol signaling pathway participated in different cancers’ liver metastases [32-34]. Holder et al. had reported gap junction may affect cancer metastases since connections are made between the primary tumor cells and foreign host cells at the secondary metastatic site [35]. Found of epimorphin activating focal adhesion kinase/extracellular signal-regulated kinase/matrix metalloproteinase-9 axis to promote hepatocellular carcinoma invasion and metastases verified focal adhesion and ECM-receptor interaction’s role in liver metastases. For this, we have reasons to believe the other seemingly irrelevant pathways would have their functions in uveal melanoma metastases.

Investigating genes involved in significant GOs and pathways, 297 genes in common were found that may affect the liver metastases of uveal melanoma patients. YWHAZ and FYN were identified as key genes in uveal melanoma liver metastases and play crucial roles in cell proliferation, which may be related to the higher incidence of metastases in uveal melanoma. YWHAZ previously was thought as a reference gene in many cell lines,
recently it was found to play a major role in YWHAZ/ beta-Catenin Axis and promote Epithelial-Mesenchymal Transition and Lung Cancer Metastases [36,37]. FYN was a member of the Src family of kinases and though lack of data about its role in uveal melanoma, it participated in many cancers metastases through different pathways [38-40]. Based on these data, further studies of these genes’ expression and the protein functions of HTR2B, CHL1, the ZNF family, YWHAZ and FYN need to be performed in more samples using reverse transcriptase-polymerase chain reaction and western blotting; moreover, for the GO and pathway findings are based on the currently known database, the regulation of identified genes and protein functions may have some other ways.

The above results all suggest that differences in gene expression exist between liver metastases and non liver metastases uveal melanoma. These genes encode proteins involved in different GOs and signal pathways, the disruption of which can promote cancer metastases. Several genes, such as HTR2B, CHL1, the ZNF family, YWHAZ and FYN provide potential candidates for distinguishing between uveal melanoma whether contain liver metastases in the future. This distinction will aid in the diagnosis and prevention of uveal melanoma liver metastases, based on their different features. Therefore, our results may provide important referential merit for clinical investigation. Nevertheless, the genes and the related GOs and pathways identified here are required to be further dissected and confirmed in more patient samples by other clinic-related studies.

Conclusion
Bioinformatics may help excavate and analyze large amounts of data in microarrays by means of rigorous experimental planning, scientific statistical analysis and collection of complete data about liver metastases of uveal melanoma patients. In the present study, a novel differential gene expression pattern was constructed and advanced study will provide new targets for diagnosis and mechanism of uveal melanoma liver metastases.

Authors’ contributions
YZ carried out the molecular genetic studies, participated in the gene alignment and drafted the manuscript. YY participated in the design of the study and performed the statistical analysis. LC participated in the gene alignment and its design and coordination. JZ conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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