

RESEARCH ARTICLE

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# 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 intracocular 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 metastases [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 histopathological features have been correlated with survival, including patient age (>60), anterior location of the tumor,

tumor cell histology, largest diameter of the tumor, mitotic 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 development of more effective local therapies for primary tumors, the rate of metastatic death remains unchanged. Unfortunately, once uveal melanoma has spread to distant 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 advance 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 throughput, new

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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

**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
Extrascleral 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.

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  $\chi^2$  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.

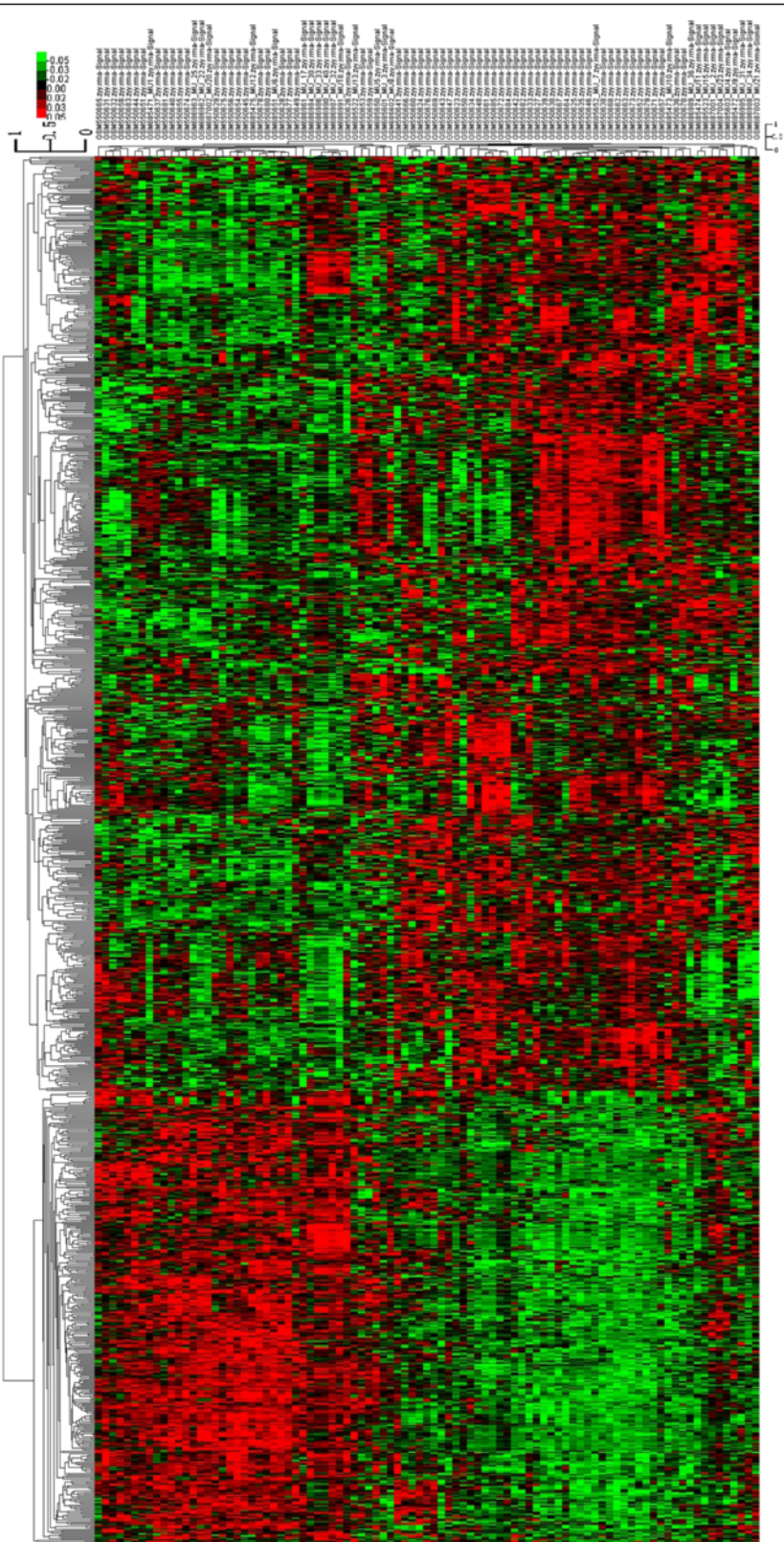


Figure 1 (See legend on next page.)

(See figure on previous page.)

**Figure 1 Unsupervised classification of uveal melanoma samples based on gene expression profiling.** Classification of 91 uveal melanoma samples using the 1138-probe sets identified as differentially expressed between the 46 liver metastatic samples and the 45 non-metastatic samples. Expression data are depicted as a data matrix where each row represents a gene and each column represents a sample. Expression levels are depicted according to the color scale shown at the top. Red and green indicate expression levels, respectively, above and below the median. The magnitude of deviation from the median is represented by the color saturation.

### 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.

**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	$1.67 \times 10^{-9}$	51.47	88.42	1.72
EXT1	$2.82 \times 10^{-9}$	203.15	362.35	1.78
DERL1	$4.89 \times 10^{-9}$	178.31	412.54	2.31
GALNTL4	$1.22 \times 10^{-8}$	57.44	128.49	2.24
MR1	$1.41 \times 10^{-8}$	60.08	98.74	1.64
DDX39	$3.31 \times 10^{-8}$	347.31	556.46	1.60
HTR2B	$3.49 \times 10^{-8}$	41.04	574.49	14.00
RAB2A	$5.29 \times 10^{-8}$	363.13	741.99	2.04
C10orf26	$6.68 \times 10^{-8}$	376.40	569.30	1.51
GJC1	$7.39 \times 10^{-8}$	25.95	75.05	2.89
CHL1	$8.99 \times 10^{-8}$	80.84	17.12	0.21
ZNF33B	$2.17 \times 10^{-8}$	41.91	21.24	0.51
OVOS2	$2.44 \times 10^{-8}$	857.96	185.87	0.22
EIF1B	$3.35 \times 10^{-7}$	1008.84	567.87	0.56
PHLDA1	$5.22 \times 10^{-7}$	370.24	134.93	0.36
PLSCR4	$5.34 \times 10^{-7}$	114.83	53.41	0.47
MEGF10	$8.38 \times 10^{-7}$	165.39	26.05	0.16
ZNF415	$1.19 \times 10^{-6}$	37.03	13.91	0.38
ZNF667	$1.25 \times 10^{-6}$	34.08	16.02	0.47
MEGF10	$1.66 \times 10^{-6}$	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).

### 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 ( $\pm$  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 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	$3.49 \times 10^{-8}$	5.36	9.17	14.00
PPM1K	$2.96 \times 10^{-6}$	7.67	9.39	3.30
SSX4	$1.03 \times 10^{-4}$	5.65	7.28	3.09
PTGER4	$3.01 \times 10^{-3}$	6.75	8.36	3.06
MEGF10	$8.38 \times 10^{-7}$	7.37	4.70	0.16
SYNPR	$1.25 \times 10^{-5}$	9.15	6.83	0.20
CHL1	$8.99 \times 10^{-8}$	6.34	4.10	0.21
OVOS2	$2.44 \times 10^{-7}$	9.74	7.54	0.22
LOC100128252	$5.70 \times 10^{-6}$	7.54	5.55	0.25
MPPED2	$5.58 \times 10^{-6}$	6.28	4.40	0.27
CNTN3	$1.37 \times 10^{-5}$	5.40	3.60	0.29
PCDH20	$2.94 \times 10^{-3}$	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).

**Table 4 Different gene significant upregulated GO**

GO item	Fold enrichment	P value
Germ cell programmed cell death	32.29	$2.80 \times 10^{-3}$
Developmental programmed cell death	19.37	$8.95 \times 10^{-3}$
Protein retention in ER lumen	13.84	$1.80 \times 10^{-2}$
Germ cell migration	13.45	$3.65 \times 10^{-4}$
Melanocyte differentiation	9.22	$8.27 \times 10^{-3}$
Mitotic cell cycle spindle assembly checkpoint	8.81	$4.36 \times 10^{-2}$
Negative regulation of mitotic metaphase/anaphase transition	8.81	$4.36 \times 10^{-2}$
Pigmentation during development	8.69	$1.14 \times 10^{-4}$
Pigment cell differentiation	8.61	$1.01 \times 10^{-2}$
Antigen processing and presentation of peptide antigen via MHC class I	7.60	$1.44 \times 10^{-2}$
Positive regulation of calcium ion transport into cytosol	6.46	$2.26 \times 10^{-2}$
Regulation of calcium ion transport into cytosol	5.57	$1.16 \times 10^{-2}$
T cell proliferation	4.78	$4.95 \times 10^{-2}$
Regulation of transforming growth factor beta receptor signaling pathway	4.14	$3.15 \times 10^{-2}$
Positive regulation of homeostatic process	4.04	$3.42 \times 10^{-2}$
Response to vitamin A	3.84	$4.00 \times 10^{-2}$
Pigmentation	3.83	$9.50 \times 10^{-3}$
Mitotic cell cycle checkpoint	3.75	$4.30 \times 10^{-2}$
Regulation of calcium ion transport	3.74	$5.31 \times 10^{-3}$
Extracellular matrix organization	3.73	$3.63 \times 10^{-4}$
Alcohol biosynthetic process	3.67	$4.62 \times 10^{-2}$
Regulation of metal ion transport	3.59	$3.49 \times 10^{-3}$
Leukocyte mediated immunity	3.38	$5.04 \times 10^{-3}$
Cellular response to insulin stimulus	3.32	$1.83 \times 10^{-2}$
Regulation of ion transport	3.26	$3.51 \times 10^{-3}$
Lymphocyte mediated immunity	3.23	$2.09 \times 10^{-2}$
Response to organic cyclic substance	3.20	$1.30 \times 10^{-3}$
Response to toxin	3.18	$3.98 \times 10^{-2}$
Double-strand break repair	3.12	$4.23 \times 10^{-2}$
Sphingolipid metabolic process	3.01	$2.82 \times 10^{-2}$
Response to molecule of bacterial origin	3.00	$1.70 \times 10^{-2}$
Adaptive immune response	2.94	$3.16 \times 10^{-2}$
Response to lipopolysaccharide	2.94	$3.16 \times 10^{-2}$
Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	2.94	$3.16 \times 10^{-2}$
Cellular response to hormone stimulus	2.91	$2.77 \times 10^{-3}$
Response to insulin stimulus	2.91	$1.22 \times 10^{-2}$
Membrane lipid metabolic process	2.79	$3.91 \times 10^{-2}$
Activation of MAPK activity	2.76	$4.11 \times 10^{-2}$

**Table 4 Different gene significant upregulated GO (Continued)**

GO item	Fold enrichment	P value
Immune effector process	2.65	$8.66 \times 10^{-3}$
Protein processing	2.59	$2.27 \times 10^{-2}$
Extracellular structure organization	2.57	$4.72 \times 10^{-3}$
Response to oxidative stress	2.56	$4.95 \times 10^{-3}$
Positive regulation of MAP kinase activity	2.53	$3.86 \times 10^{-2}$
Regulation of MAP kinase activity	2.52	$1.21 \times 10^2$
Amine transport	2.46	$2.99 \times 10^{-2}$
Camera-type eye development	2.41	$4.79 \times 10^{-2}$
Carbohydrate biosynthetic process	2.41	$4.79 \times 10^{-2}$
Protein oligomerization	2.41	$7.85 \times 10^{-3}$
Protein maturation	2.38	$3.54 \times 10^{-2}$
Nucleotide biosynthetic process	2.26	$1.29 \times 10^2$
Nucleobase, nucleoside and nucleotide biosynthetic process	2.17	$1.69 \times 10^{-2}$
Nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process	2.17	$1.69 \times 10^{-2}$
Cell proliferation	2.15	$2.12 \times 10^{-4}$
Muscle organ development	2.14	$1.41 \times 10^2$
Regulation of neurogenesis	2.14	$3.34 \times 10^{-2}$
Skeletal system development	2.13	$2.21 \times 10^{-3}$
Response to peptide hormone stimulus	2.10	$4.96 \times 10^{-2}$
Transmembrane receptor protein tyrosine kinase signaling pathway	2.02	$2.20 \times 10^{-2}$
Regulation of nervous system development	2.02	$3.63 \times 10^{-2}$
Nitrogen compound biosynthetic process	1.99	$5.98 \times 10^{-3}$
Enzyme linked receptor protein signaling pathway	1.98	$4.86 \times 10^{-3}$
Response to drug	1.94	$3.62 \times 10^{-2}$
Blood vessel development	1.84	$4.11 \times 10^{-2}$
Response to DNA damage stimulus	1.82	$1.20 \times 10^{-2}$
Induction of apoptosis	1.82	$2.14 \times 10^{-2}$
Induction of programmed cell death	1.81	$2.19 \times 10^{-2}$
Positive regulation of apoptosis	1.80	$7.74 \times 10^{-3}$
Vasculature development	1.80	$4.83 \times 10^{-2}$
Positive regulation of programmed cell death	1.79	$8.09 \times 10^{-3}$
Positive regulation of cell death	1.78	$8.74 \times 10^{-3}$
M phase	1.77	$2.70 \times 10^{-2}$
Response to hormone stimulus	1.76	$1.98 \times 10^{-2}$
Response to endogenous stimulus	1.75	$1.46 \times 10^{-2}$
Cell cycle phase	1.72	$1.82 \times 10^{-2}$
Response to abiotic stimulus	1.67	$3.73 \times 10^{-2}$
Protein kinase cascade	1.66	$3.89 \times 10^{-2}$
Cellular response to stress	1.65	$9.67 \times 10^{-3}$
Response to organic substance	1.61	$5.45 \times 10^{-3}$

**Table 4 Different gene significant upregulated GO (Continued)**

Phosphorylation	1.57	$5.41 \times 10^{-3}$
Oxidation reduction	1.57	$1.51 \times 10^{-2}$
Phosphate metabolic process	1.56	$2.49 \times 10^{-3}$
Phosphorus metabolic process	1.56	$2.49 \times 10^{-3}$
Cell cycle process	1.54	$2.79 \times 10^{-2}$
Protein amino acid phosphorylation	1.50	$2.49 \times 10^{-2}$
Immune response	1.50	$2.35 \times 10^{-2}$
Regulation of cell proliferation	1.44	$3.02 \times 10^{-2}$
Regulation of programmed cell death	1.43	$2.88 \times 10^{-2}$
Regulation of cell death	1.43	$3.01 \times 10^{-2}$
Regulation of apoptosis	1.41	$3.91 \times 10^{-2}$

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 5 Different gene significant downregulated GO**

GO item	Fold enrichment	P value
Calcium-dependent cell-cell adhesion	9.49	$3.90 \times 10^{-2}$
Response to acid	9.09	$4.22 \times 10^{-2}$
Embryonic skeletal system development	5.67	$4.04 \times 10^{-3}$
Response to vitamin	5.51	$1.26 \times 10^{-2}$
Regulation of cell shape	5.39	$3.75 \times 10^{-2}$
Regulation of Rho protein signal transduction	5.14	$2.36 \times 10^{-3}$
Pigmentation	4.93	$4.68 \times 10^{-2}$
Cartilage development	4.91	$1.85 \times 10^{-2}$
Skeletal system morphogenesis	3.90	$1.88 \times 10^{-2}$
Regulation of cell morphogenesis	3.89	$9.22 \times 10^{-3}$
Translational elongation	3.60	$4.97 \times 10^{-2}$
Muscle tissue development	3.49	$2.85 \times 10^{-2}$
Homophilic cell adhesion	3.33	$3.40 \times 10^{-2}$
Regulation of neuron differentiation	3.28	$3.59 \times 10^{-2}$
Embryonic organ morphogenesis	3.28	$3.59 \times 10^{-2}$
Regulation of cell development	3.19	$7.23 \times 10^{-3}$
Regulation of Ras protein signal transduction	3.12	$8.25 \times 10^{-3}$
Muscle organ development	3.10	$8.54 \times 10^{-3}$
Regulation of neurogenesis	3.07	$2.66 \times 10^{-2}$
Response to drug	3.03	$9.75 \times 10^{-3}$
Embryonic organ development	2.96	$3.09 \times 10^{-2}$
Regulation of small GTPase mediated signal transduction	2.89	$7.88 \times 10^{-3}$
Skeletal system development	2.74	$4.38 \times 10^{-3}$
Heart development	2.71	$2.85 \times 10^{-2}$
Regulation of nervous system development	2.65	$4.85 \times 10^{-2}$
Cell-cell adhesion	2.64	$1.37 \times 10^{-2}$
Negative regulation of cell proliferation	2.62	$4.07 \times 10^{-3}$
Cell adhesion	2.60	$2.99 \times 10^{-5}$
Biological adhesion	2.59	$3.06 \times 10^{-5}$
Actin cytoskeleton organization	2.57	$3.59 \times 10^{-2}$
Actin filament-based process	2.41	$4.79 \times 10^{-2}$
Chordate embryonic development	2.20	$3.87 \times 10^{-2}$
Embryonic development ending in birth or egg hatching	2.18	$4.03 \times 10^{-2}$
Cytoskeleton organization	2.17	$1.68 \times 10^{-2}$
Regulation of cell proliferation	2.03	$2.54 \times 10^{-3}$
Response to organic substance	1.71	$3.85 \times 10^{-2}$

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.

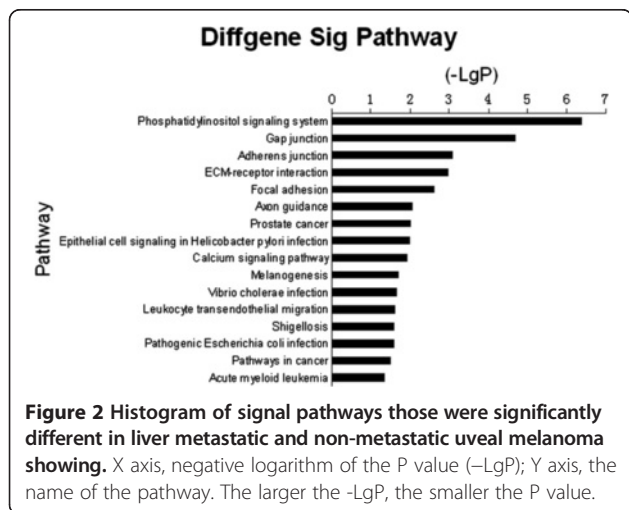
### Discussion

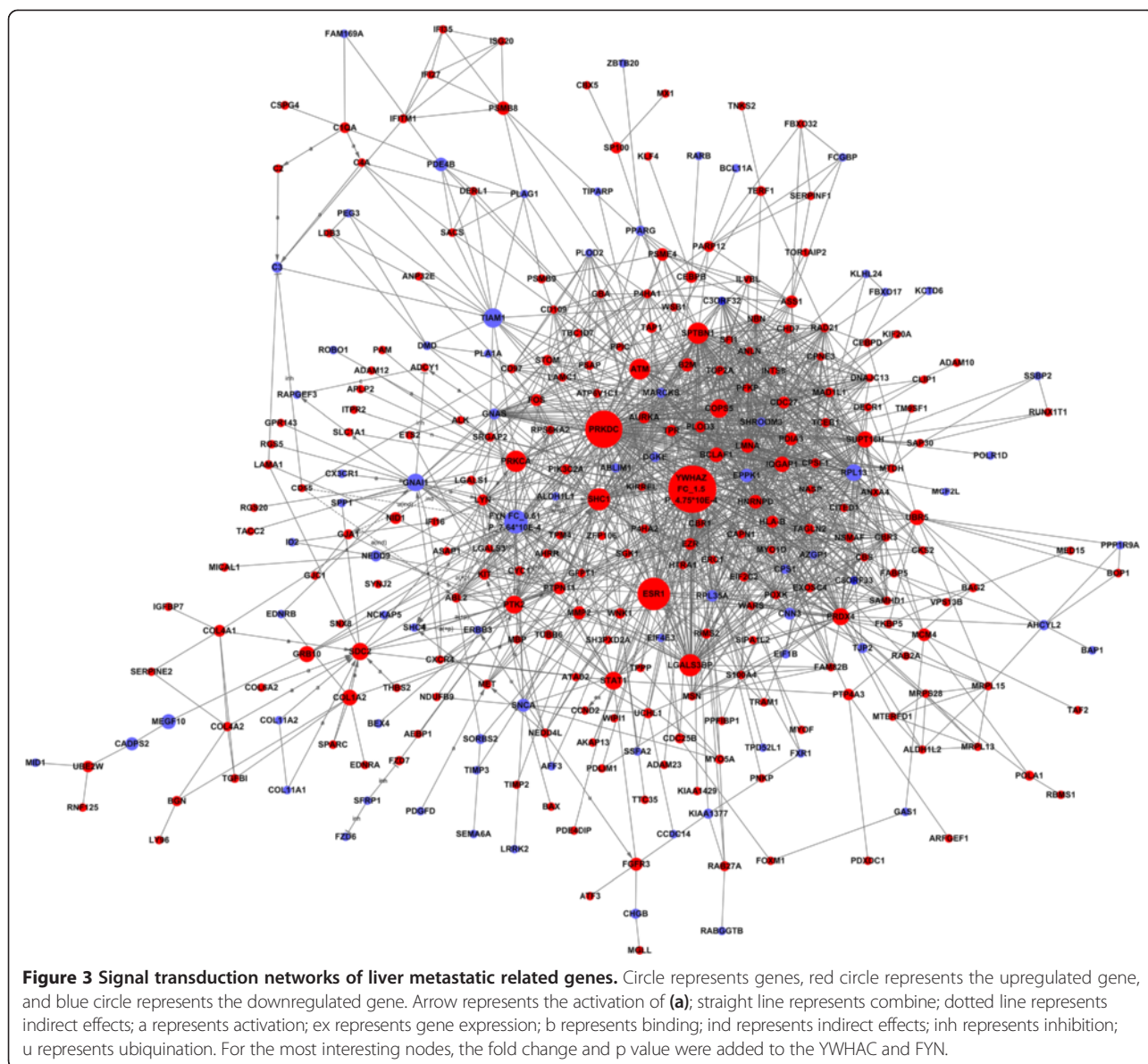
The present study followed up microarray-based 91 uveal melanoma patients, of whom patients were not significantly different in age, gender and tumor diameter. More than 1100 probes were differentially dysregulated in liver metastases uveal melanoma in this study. Based on sorting the different genes 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 the largest upregulated fold change in the two groups.

Soll et al. had found serotonin can promote tumor growth in hepatocellular cancer [24] and this maybe an effective hint to us. CHL1 is a cell adhesion molecule with homology to L1CAM involved in the regulation of cell adhesion and migration. Current study had supported a significant role for CHL1 gene in the growth, migration and invasion of human cervical cancer cells [25]. MEGF10 and OVOS2 have the largest downregulated fold change and a significant p value. It is still lack of information about their roles both in tumor and ophthalmology. Zinc finger proteins are among the most abundant proteins which were involved in transcriptional activation, apoptosis regulation and protein folding. Some zinc finger protein had been detected related to melanoma [26]. However, there have been no reports of a differential expression of ZNF family members in liver metastases uveal melanoma. Comparing with the original papers of the expression microarray, we found PTP4A3/PRL3 still has significant difference while SDCBP dropped out with too little fold change. In our opinion, this is caused by the enlargement of sample number which may impact larger in the previously smaller sample group for GSE27831 has only 29 samples. For the sample number we integrate here is not so small, though these genes were still lack of information about their impact on uveal melanoma liver metastases, we have the reason to focus on them in further investigation.

The GO is widely recognized as the premier tool for the organization and functional annotation of molecular aspect [27]. GO-analysis was used to interpret each GO of differential expressed gene and analyzed it statistically. By using the criteria of  $P < 0.05$ , we obtained the significant GOs and genes involved in them. Guo et al. used GO-analysis to analyze miRNA microarray and found that miR-15b and miR-16 may be indispensable for apoptosis by targeting Bcl-2 [28]. GO terms about programmed cell death plays an important role in liver metastases, many studies had reported proteins such as TRAIL and TGF- $\beta$ 1 involved in cell apoptosis also affected cancer cell metastases [29,30]. Ion transport related genes is little investigated in cancer cell metastases, but recently Lee et al. found monoamine carboxylate transporters could impact colon fibrosarcoma cell migration through regulating extracellular pH value [31]. This may be a new way investigating uveal melanoma liver metastases. Also the structural changes of intracellular organelles and cytoskeletal would of course act on cell migration and invasion. According to our results, programmed cell death, ion transport and cytoskeletal would affect cell environment to play roles in liver metastases. Furthermore, other biological process may also have their effects in uveal melanoma distant metastases.

Pathways analysis can show the distinct biological process and find significant pathways that differential





expression genes participate in, based on which can we 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.

## Additional files

**Additional file 1: Figure S1.** Normalized microarray data by Pearson's Correlation. Uveal melanoma samples microarray data were normalized by Pearson's Correlation and one sample GSM685470 was removed.

**Additional file 2: Figure S2.** Venn diagram for different methods of gene expression analysis. T-test, SAM and RVM mode were used to screen the differential expressed genes.

**Additional file 3: Gene feature and relationship in the liver metastatic related signal transduction network.**

## Competing interests

The authors declare that they have no competing interests.

## 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.

## Acknowledgements

This study was supported by the 11th five-year plan of science and technology research projects of China (no. 2007BAI18B07).

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Received: 29 December 2012 Accepted: 12 February 2014

Published: 5 March 2014

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doi:10.1186/1471-2350-15-29

**Cite this article as:** Zhang *et al.*: Expression analysis of genes and pathways associated with liver metastases of the uveal melanoma. *BMC Medical Genetics* 2014 **15**:29.

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