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Research Article

Transcriptomic Changes Associated with Uveal Melanoma Metastasis

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Abstract

Purpose: Survival of patients affected by Uveal Melanoma (UM), a major intraocular cancer, is greatly reduced by the development of metastasis. However, the molecular tumor characteristics associated with metastasis are unclear. The purpose of this study was to discover differential gene expression among non-metastatic and metastatic UM patients and to evaluate their prognostic significance.

Methods: We utilized the UM RNA-seq dataset from The Cancer Genome Atlas (TCGA) to discover the genes associated with UM metastasis and patient survival. Differential expression analyses between metastatic and non-metastatic tumors were performed. The hazard ratios were computed to correlate differentially expressed genes with patient survival. Bioinformatics analyses were also conducted to identify associated biological functions and pathways.

Results: A total of 646 genes were differentially expressed between metastatic and non-metastatic tumors and 328 genes were significantly correlated with patient survival. The top five genes upregulated in metastasis and negatively associated with patient survival include: *HTR2B*, *RIMS2*, *VGF*, *MYEOV*, and *ISM1*. The top five genes downregulated in metastasis and positively associated with patient survival include: *GSAT3*, *GATA4*, *MYO7B*, *COL11A1*, and *SYNPR*. Functional annotation of these genes revealed a number of molecular and cellular functions including cell movement, growth, proliferation, cell junction, and transporter activity.

Conclusion: We identified several differentially expressed genes associated with metastasis in UM patients which correlated significantly with patient survival. Several genes are associated with cell movement and homeostasis, indicating their significance in metastasis. The findings from this study may aid in the development of prognostic and predictive biomarkers for metastatic UM.

Keywords: uveal melanoma, metastasis, survival, gene expression

Introduction

Uveal melanoma (UM) is the most primary intraocular malignancy, and unlike nearly all other cancers, the 5-year survival rate has not improved over the past 40 years [1, 2]. Approximately half of all UM patients eventually develop metastases - most commonly to the liver (~89%), lung (~29%), and bone (~17%) [3, 4]. Metastasis bodes poorly with the survival, with the median patient survival ranging from 4 to 15 months and one-year survival being only ~20% [5–12]. No adjuvant therapy after treatment of the primary tumor has been shown to prevent the development of metastasis [13]. Advances in molecular-targeted therapies have improved the survival of patients with Cutaneous Melanomas (CM), however, these therapies are ineffective in UM [6]. This likely is related

to different underlying mutations between UM and CM (CM candidate genes CDKN2A, p14ARF, CDK4 were either not present or observed at a low frequency in UM patients), highlighting clear molecular differences between uveal and cutaneous melanomas [14].

Several genetic alterations, including copy number variations and somatic mutations, are reported in UM [15]. The most frequent chromosomal aberrations encountered in UM are monosomy of chromosome 3 (the strongest cytogenetic factor associated with metastasis), and amplification of 8q [16–18]. The majority of metastatic UM present with a mutated allele of the BAP1, which behaves like a classic tumor suppressor [19]. The amplification on chromosome 6q25.2 near the *CNKSR3* gene prolongs metastasis-free survival in a rare subset of UM [20]. The mutations in GNAQ and GNA11, which encode for Gα subunits of G proteins, are present in over 80% of UM [21]. Amplification of these genes leads to the stimulation of several pathways, including Akt, protein kinase C (PKC), phosphoinositide 3-kinase (PI3K), and the mechanistic target of rapamycin (mTOR), which are involved in cancer cell proliferation and metastasis [21]. Given the wide range of chromosomal and genetic alterations, gene expression profiling of UM might offer valuable insights into the molecular mechanisms underlying disease pathogenesis and prognosis [15, 18, 22–31].

The rarity of uveal melanoma (incidence: ~5 per million) presents a big challenge for studies designed to identify molecular signatures associated with UM metastasis [32]. The Cancer Genome Atlas (TCGA) is a valuable resource containing gene expression data from 80 UM patients (http: //cancergenome.nih.gov/). Utilizing the high quality TCGA RNA-Seq UM dataset, we discovered differentially expressed genes between primary tumor of patients who developed metastasis and those without metastasis. Further, the prognostic potential of these gene expression changes in relation to patient survival was also evaluated.

Materials And Methods

Dataset

The uveal melanoma RNA-seq dataset was downloaded from the TCGA consortium (https: //portal.gdc.cancer.gov/). The dataset includes the expression data of 20,530 genes from 80 UM patients with accompanying clinical information on survival and metastatic status.

Differential Expression Analysis

Statistical analyses were performed using the R language and environment for statistical computing (R version 3.5.2; R Foundation for Statistical Computing; www.r-project.org). After normalization of the gene expression data, differential expression analyses were performed between primary UM tumors with and without metastasis using the *"limma"* package [33]. The p-values were adjusted using the false discovery rate (FDR) method and genes with adjusted p-values <0.01 and fold-change >2 were considered to be differentially expressed.

Survival Analysis

For each gene, subjects were separated into high- or low-expression groups relative to the median gene expression value. Cox proportional hazard models were then used to perform survival analyses. The hazard ratios (HR) were computed, and p-values were adjusted using the FDR method. Genes with adjusted p-value <0.01 were considered to be significantly associated with patient survival. Concordance was also measured to evaluate the performance of the survival models.

Pathway and Network Analyses

Genes associated with UM metastasis and patient survival were submitted to the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 [34, 35] in an attempt to deduce involved biological pathways, cellular compartments, and molecular functions. Similarly, Ingenuity Pathway Analysis (IPA) software was used for network analysis to display the interactions between these genes.

Results

Gene Expression Changes Associated with Metastasis

A total of 646 genes were differentially expressed with a 2-fold or more up- or down-regulation between the metastatic and non-metastatic subjects (adjusted p-value

<0.01). A volcano plot visualizing the results of the differential expression analysis is shown in Figure 1. The number of upregulated and downregulated genes in metastatic UM, using different Fold-Change (FC) cutoffs (2-, 3-, 4-, 5-, and 10-fold) are presented in Table 1.

Table 1: Number of genes differentially expressed in metastatic uveal melanoma at different fold-change cut-off values.

Fold-Change	Number of genes				
	>2-fold	>3-fold	>4-fold	>5-fold	>10-fold
Metastasis	646	178	76	31	3
Upregulated	469	118	48	20	2
Downregulated	177	60	28	11	1
Metastasis and Survival	328	126	56	26	3

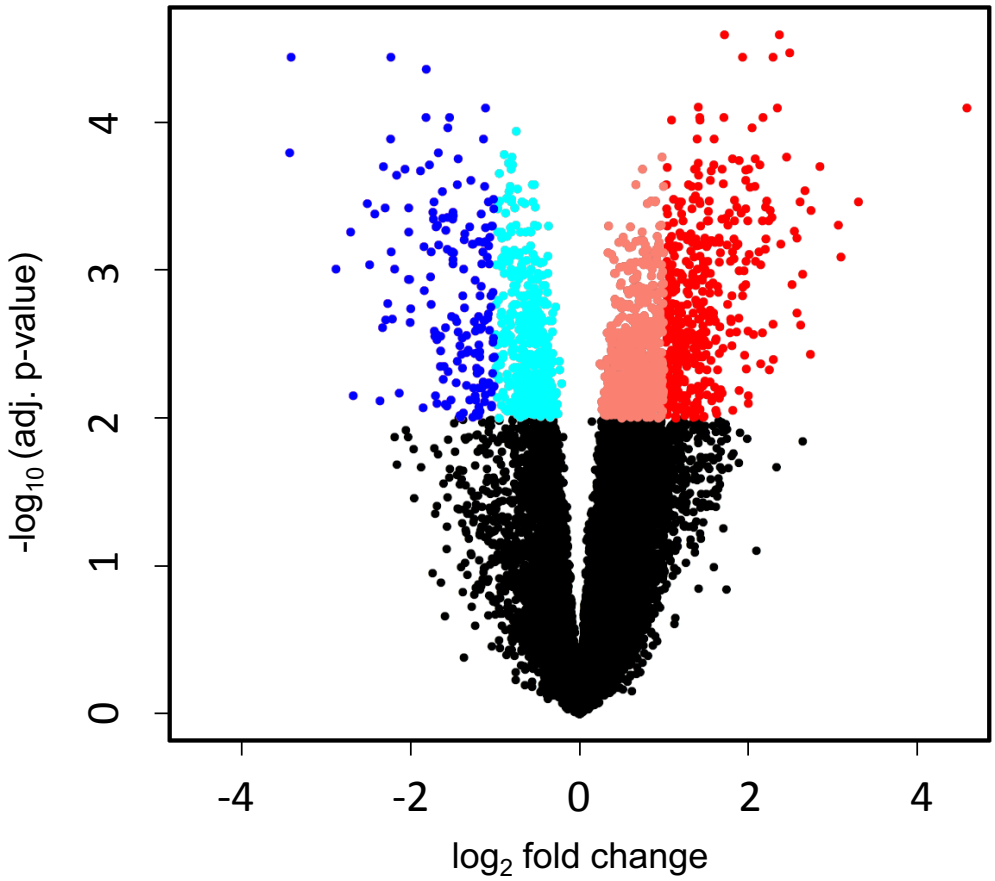

Figure 1: Volcano plot visualizing the results of differential expression analysis. Each dot represent one gene. Red dots represent significantly upregulated genes and blue dots represent significantly downregulated genes in metastatic UM.

The most upregulated genes in the metastatic UM include 5-hydroxytryptamine receptor 2B (*HTR2B*; FC: 24.1), regulating synaptic membrane exocytosis 2 (*RIMS2*; FC: 9.9), VGF nerve growth factor (*VGF*; FC: 8.6), myeloma overexpressed (*MYEOV*; FC: 8.4), isthmin-1 (*ISM1*; FC: 7.2), carbonic anhydrase-12 (*CA12*; FC: 6.7), phospholamban (*PLN*; FC: 6.7), potassium voltage-gated channel subfamily Q member 2 (*KCNQ2*; FC: 6.4), SSX family member 5 (*SSX5*; FC: 6.3), and carbonic anhydrase 8 (*CA8*; FC: 5.97) (Table 2).

Table 2: Top 20 genes upregulated in metastatic uveal melanoma.

Gene symbol	Name	FC	p-value	HR	p-value	Concordance
HTR2B	5-hydroxytryptamine receptor 2B	24.12	8.00E-05	5.85	0.007	0.69
RIMS2	Regulating synaptic membrane exocytosis 2	9.91	0.0003	6.79	0.005	0.71
VGF	VGF nerve growth factor inducible	8.58	0.0008	8.71	0.005	0.72
MYEOV	Myeloma overexpressed	8.38	0.0005	7.33	0.005	0.70
ISM1	Isthmin 1	7.22	0.0002	10.07	0.005	0.77
CA12	Carbonic anhydrase 12	6.70	0.0004	10.64	0.005	0.77
PLN	Phospholamban	6.67	0.0037	5.93	0.007	0.70
KCNQ2	Potassium voltage-gated channel Q 2	6.38	0.0003	9.12	0.005	0.72
SSX5	SSX family member 5	6.26	0.0011	17.33	0.005	0.75
CA8	Carbonic anhydrase 8	5.97	0.0006	4.44	0.008	0.70
SLCO5A1	Solute carrier organic anion transporter 5A1	5.84	0.0005	4.90	0.008	0.71
ST8SIA2	ST8 sialyltransferase 2	5.63	3.36E-05	14.13	0.005	0.74
DOCK10	Dedicator of cytokinesis 10	5.48	0.0002	5.44	0.008	0.70
MATK	Megakaryocyte-associated tyrosine kinase	5.23	0.0007	5.91	0.005	0.72
SLC1A1	Solute carrier family 1 member 1	5.17	2.54E-05	9.65	0.005	0.75
PSD2	Pleckstrin and Sec7 domain containing 2	5.09	8.00E-05	6.77	0.005	0.74
CADM1	Cell adhesion molecule 1	4.91	3.62E-05	11.01	0.005	0.76
EEF1A2	Eukaryotic translation elongation factor 1α2	4.91	0.0023	5.11	0.007	0.67
CARD11	Caspase recruitment domain family 11	4.86	0.0004	18.92	0.005	0.78
TNFRSF19	TNF receptor superfamily member 19	4.79	0.0004	10.13	0.005	0.76

FC: Fold change; HR: Hazards Ratio

The most downregulated genes in patients with UM metastasis include glutathione S-transferase alpha 3 (*GSTA3*; FC: 10.8), GATA binding protein 4 (*GATA4*; FC: 10.7), myosin VIIB (*MYO7B*; FC: 7.4), collagen type XI alpha 1 chain (*COL11A1*; FC: 6.6), synaptoporin (*SYNPR*; FC: 6.4), cytochrome c oxidase subunit 6A2 (*COX6A2*; FC: 5.4), musculin (*MSC*; FC: 5.04), cardiotrophin-1 (*CTF1*; FC: 5), embryonal Fyn-associated substrate (*EFS*; FC: 4.93), interleukin 12 receptor subunit beta 2 (*IL12RB2*; FC: 4.84), and ectonucleotide pyrophosphatase (*ENPP2*: FC: 4.73) (Table 3).

Gene Expression Changes Correlated with Patient Survival

Considering the results of the Cox proportional hazard analysis of 646 differentially expressed genes 328 were significantly correlated to patient survival. Out of 76 genes with >4-fold change between non-metastatic and metastatic cases, 56 significantly correlated with patient survival. A heatmap representing gene expression values of these 56 genes in all 80 patients is shown in Figure 2.

Table 3: Top 20 genes downregulated in metastasis developing uveal melanoma.

Symbol	Name	FC	p value	HR	p value	Concordance
GSTA3	Glutathione S-transferase alpha 3	-10.81	0.0002	0.07	0.005	0.77
GATA4	GATA binding protein 4	-10.71	3.62E-05	0.04	0.008	0.74
MYO7B	Myosin VIIB	-7.40	0.0010	0.18	0.008	0.70
COL11A1	Collagen type XI alpha 1 chain	-6.56	0.0006	0.17	0.005	0.74
SYNPR	Synaptoporin	-6.42	0.0070	0.13	0.005	0.68
C6orf142	Muscular LMNA interacting protein	-5.73	0.0004	0.09	0.005	0.76
LOC100188947	Uncharacterized antisense RNA	-5.61	0.0009	0.13	0.006	0.73
COX6A2	Cytochrome c oxidase subunit 6A2	-5.38	0.0004	0.11	0.005	0.72
MSC	Musculin	-5.04	0.0025	0.14	0.005	0.69
CTF1	Cardiotrophin 1	-5.01	0.0002	0.09	0.005	0.75
EFS	Embryonal Fyn-associated substrate	-4.93	0.0004	0.05	0.005	0.77
C3orf32	Ssu-2 homolog (C. elegans)	-4.92	0.0022	0.14	0.005	0.76
IL12RB2	Interleukin 12 receptor subunit β2	-4.84	0.0017	0.07	0.005	0.75
ENPP2	Ectonucleotide pyrophosphatase	-4.73	0.0001	0.09	0.005	0.76
LIMS2	LIM zinc finger domain	-4.71	3.62E-05	0.15	0.005	0.75
MPZ	Myelin protein zero	-4.70	0.0008	0.08	0.005	0.78
ZNF835	Zinc finger protein 835	-4.56	0.0010	0.03	0.005	0.77
AZGP1	Alpha-2-glycoprotein 1	-4.50	0.0002	0.10	0.005	0.73
ERVFRDE1	Endogenous retrovirus grp FRD1	-4.19	0.0002	0.09	0.006	0.75
CLEC11A	C-type lectin domain 11A	-4.07	0.0004	0.06	0.005	0.75

The top 20 genes upregulated in UM with metastasis and negatively correlated with patient survival are listed in Table 2. Similarly, the top 20 genes downregulated in metastasis development and positively correlated with patient survival are listed in Table 3. Survival plots for the top 10 genes, whose higher expression is associated with poor patient survival, are shown in Figure 3A. Similarly, the top 10 genes whose lower expression is associated with poor survival are shown in Figure 4A. The box plots visualizing their expression levels in metastasis development groups are shown in Figures 3B (upregulated) and Figure 4B (downregulated).

Bioinformatics Analyses

Gene ontology enrichment analyses were performed using DAVID to gain further insight into the biological functions of the 328 genes associated with UM metastasis and patient survival. The most enriched biological processes include negative regulation of apoptosis (16 genes), negative regulation of cell proliferation (12 genes), and angiogenesis (11 genes). The significantly enriched cellular components include the plasma membrane (90 genes), extracellular space (40 genes), and cell junction (14 genes) (Table 4). The enriched molecular functions include transporter activity (9 genes), lipid binding (7 genes), and antigen binding (6 genes). Overall, gene ontology enrichment analyses revealed six annotation clusters of biological functions highly enriched in the genes associated with UM metastasis. These clusters include membrane protein (132 genes), glycoprotein signaling (100 genes), cell junction (22 genes), and pleckstrin homology domain (10 genes), antigen binding: MHC class-I (5 genes), and cell communication (5 genes) (Table 4).

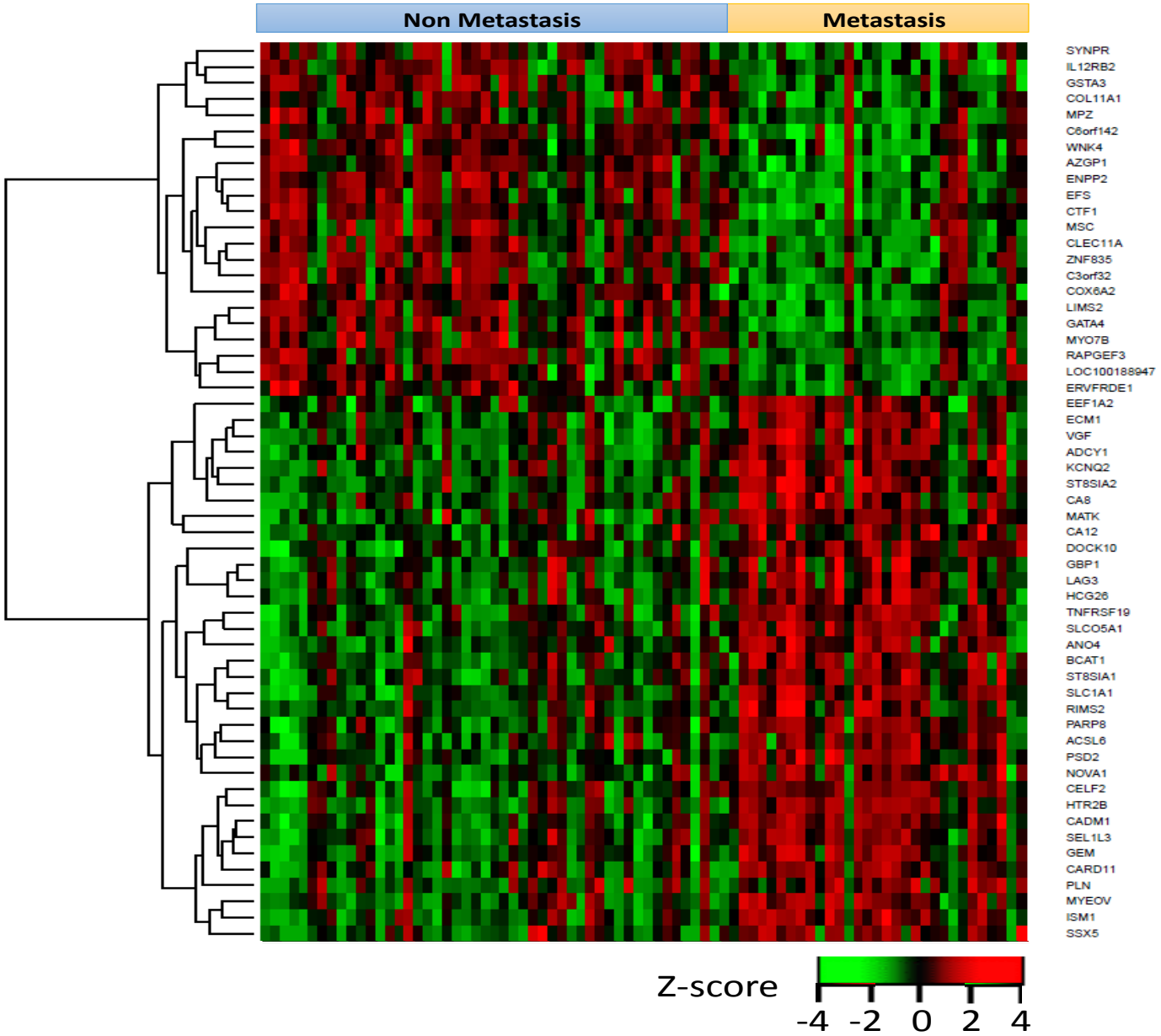

Figure 2: Heatmap representing gene expression values of these 56 genes in all 80 patients. Each row represents a gene and each column represents a patient. Red: high expression. Green: low expression.

Network Analyses

Ingenuity Pathway Analysis (IPA) software was used to discover the interactions between the genes associated with UM metastasis (Figure 5). IPA analyses also revealed that interaction network includes several genes known to be involved epithelial neoplasm, cell movement, migration of cells, development of malignant tumor, invasion of cells, gap junction signaling, endocannabinoid neuronal synapse pathway, CDK5 Signaling, endothelin-1 signaling, GABA receptor signaling, and antigen presentation pathway. The key hub genes of the network are *ERBB3* (13 connections), *PTGS2* (11 connections), *GATA4* (11 connections), *GATA6* (9 connections), *MYH7* (8 connections), and *MYCN* (8 connections). The genes in the network are grouped using their cellular compartments including extra cellular space, plasma membrane, cytoplasm, and nucleus.

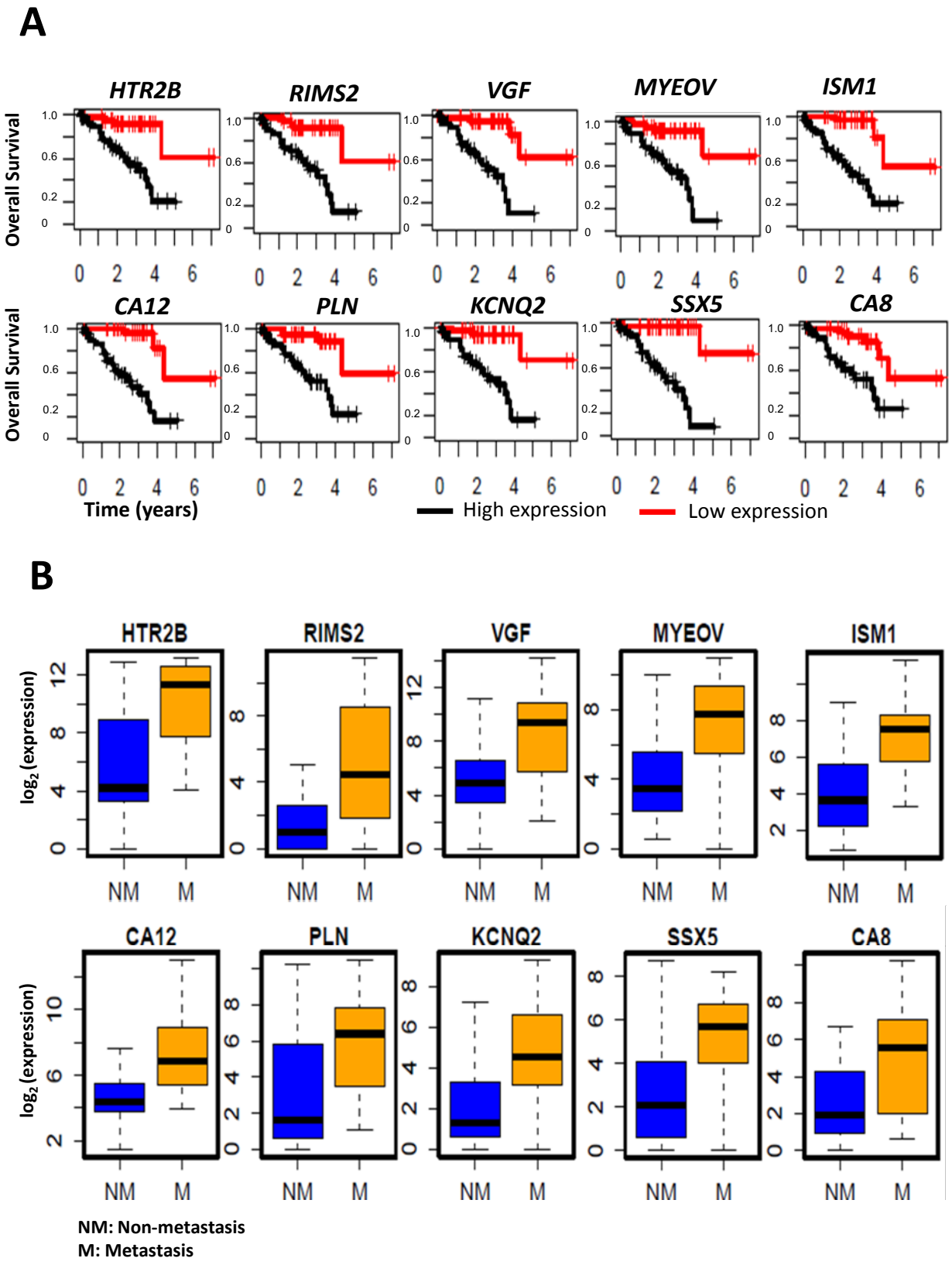

Figure 3: Upregulated genes in UM metastasis.

A: Kaplan-Meier survival curves showing a major difference in overall survival, with respect to high vs. low gene expression levels. **B:** Boxplots showing the distribution of the gene expression levels in patients with metastasis compared to patients without metastasis.

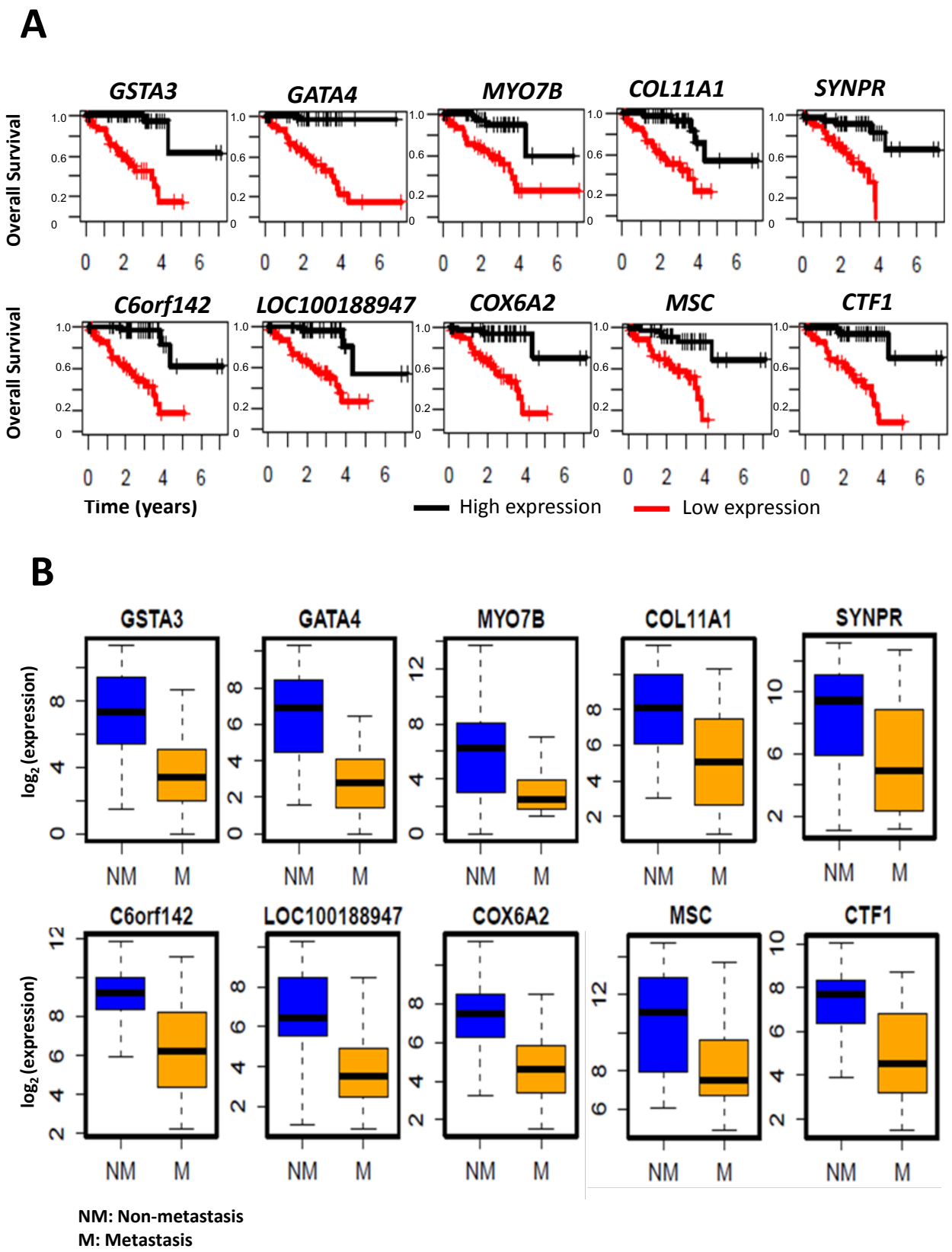

Figure 4: Downregulated genes in UM metastasis.

A: Kaplan-Meier survival curves showing a major difference in overall survival, with respect to high vs. low gene expression levels. **B:** Boxplots showing the distribution of the gene expression levels in patients with metastasis compared to patients without metastasis.

Table 4: Gene ontology groups enriched in the 328 genes associated with UM metastasis.

Gene Ontology Term	Count	p-value
Biological Processes		
Negative regulation of apoptotic process	16	0.004
Negative regulation of cell proliferation	12	0.040
Transport	11	0.041
Chemical synaptic transmission	11	0.003
Angiogenesis	11	0.002
Axon guidance	7	0.035
Regulation of cell migration	6	0.005
Protein glycosylation	6	0.029
Cell communication	5	0.002
Antigen processing and presentation	5	0.009
Cellular Components		
Plasma membrane	90	0.001
Extracellular space	40	0.009
Cell junction	14	0.019
Molecular Functions		
Transporter activity	9	0.012
Lipid binding	7	0.028
Antigen binding	6	0.020
Annotation Clusters		
Membrane Protein	132	<0.01
Glycoprotein signaling	100	<0.01
Cell Junction	22	<0.01
Protein Recognition domain: Pleckstrin homology domain	10	<0.01
Antigen Binding: MHC class I	5	<0.01
Cell communication	5	<0.01

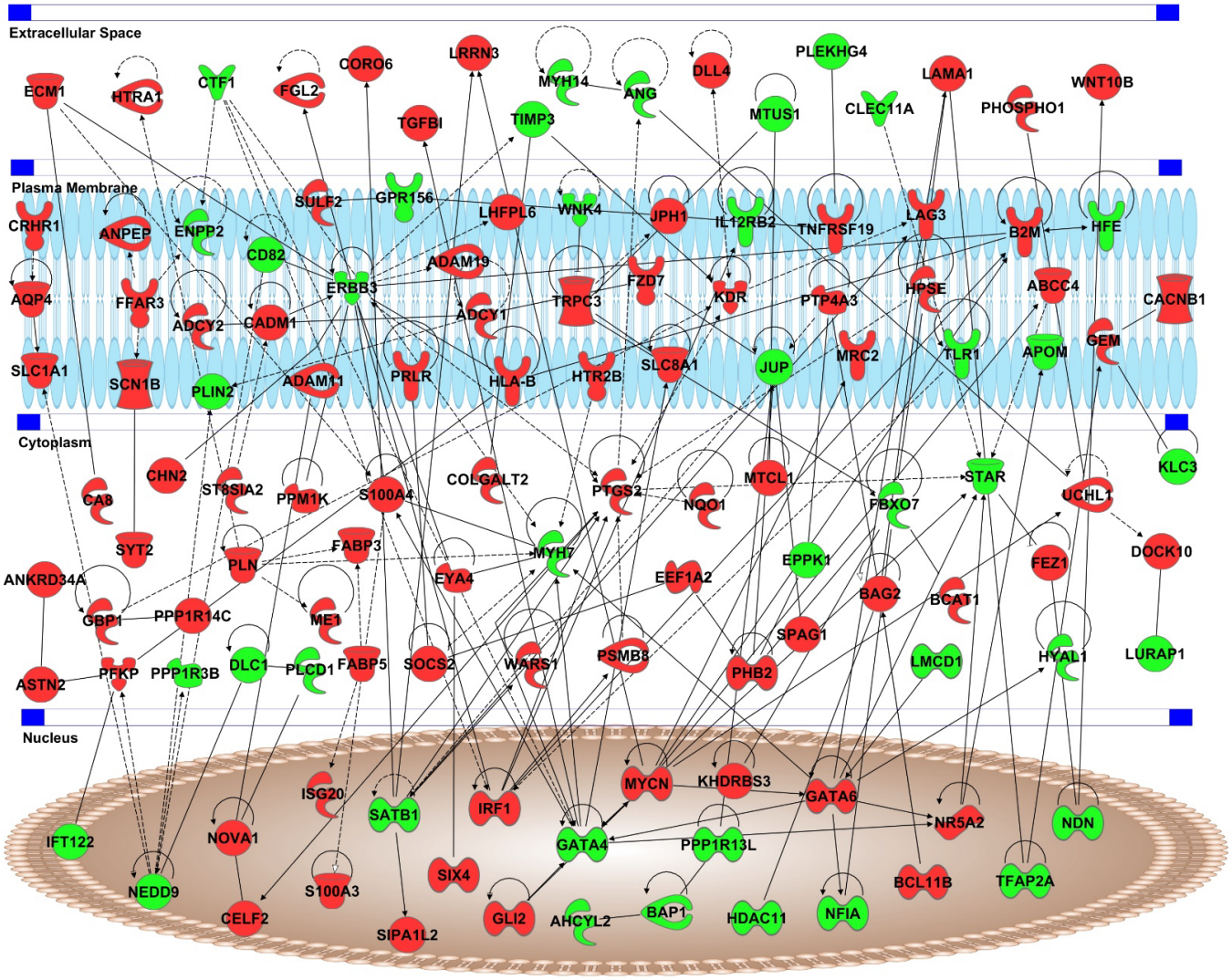

Figure 5: Interaction network of genes in associated with UM metastasis. Red color represent genes upregulated in UM metastasis and green color represents downregulated genes. Genes are separated based on the cellular compartments.

Discussion

The development of metastases plays an important role in UM patient survival. In this study, we identified gene expression signatures associated with metastasis and poor survival in UM patients. Identification of molecular characteristics associated with UM metastasis has the potential to elucidate precise molecular mechanisms, accurately classify high-risk patients, and to provide novel potential therapeutic targets.

Out of the top 20 genes found to be upregulated in metastatic UM, 12 genes including *HTR2B, RIMS2*, *VGF*, *MYEOV*, *ISM1*, *CA12*, *PLN*, *SSX5*, *SLCO5A1, SLC1A1, CADM1,* and *TNFRSF19,* have been previously reported to be associated with aggressive UM. *HTR2B* gene, which encodes for the Serotonin receptor 5-hydroxytrytamine receptor-2B, was the most upregulated gene (~24-fold) in metastatic patients, and its increased expression was also associated with poor survival. This gene is well-known to be elevated in cases of metastatic UM and is one of the genes used in the DecisionDx-UM prognostic test [36–38]. *RIMS2* encodes for regulating synaptic membrane exocytosis-2 protein involved in neuronal synaptic transmission and neurotransmitter release; mutations in *RIMS2* have been linked to inherited retinal disease. *RIMS2* and phospholamban (*PLN*) are known to be overexpressed in monosomy 3 UM [39, 40]. Phospholamban is an important regulator of cardiac muscle contractility, and its role in UM remains unclear [41]. Isthmin-1 (ISM1) is a known inhibitor of angiogenesis [42]. Interestingly,

isthmin-1 expression is regulated by long non-coding RNA (lncRNA) H19, a well-known lncRNA associated with invasion and metastasis in several cancer types [43, 44]. Carbonic anhydrase 12 (CA12), is highly expressed in many cancers and is thought to contribute to the acidification of the tumor microenvironment [45]. Solute carrier transporter family members *SLCO5A1* and *SLC1A1* are expressed by several cell types within the retina. Although these transporters are known to be overexpressed in several cancers, their role in UM has not been fully characterized [46–48]. The protein product of the *VGF* gene is a neuroprotective growth factor and has been previously identified in a proteomic profiling study of UM as being highly upregulated in high-risk UM [49, 50]. Myeloma overexpressed gene *(MYEOV)* is known to be overexpressed in UM and several other cancer types [51]. In non-small cell lung cancer, this gene has been shown to increase metastasis through the amplification of TGF-β signaling, but its function in UM has not been established [51, 52]. The transcriptional repressor synovial sarcoma X breakpoint protein 5 (SSX5) is known to be upregulated in UM, and expression of the SSX family of proteins is correlated with a more aggressive tumor phenotype *in vitro* [53]. Both *CADM1* (cell adhesion molecule 1) and *TNFRSF19* are known to be upregulated in monosomy 3 UM [39]. *TNFRSF19* is a member of the tumor necrosis factor receptor superfamily known to be regulated by beta-catenin. It has been shown to activate NF-kB, and its increased expression is linked with cell migration and invasion [54, 55].

There were 8 other genes newly identified as highly upregulated in metastatic UM. These included the following: *KCNQ2, CA8, ST8SIA2, DOCK10, MATK, PSD2, EEF1A2,* and *CARD11. KCNQ2* encodes for a voltage-gated potassium channel, and mutations in this gene are known to cause inherited neonatal epilepsy [56]. In highly metastatic breast cancer, increased expression of *KCNQ2* has been reported in conjunction with decreased expression of Na-K ATPase and may be responsible for increased potassium flux and an alternative mechanism for intracellular ion homeostasis [57]. Carbonic anhydrase-related protein-8 (*CA8)* is a carbonic anhydrase isoform that lacks conventional enzymatic activity. This protein has high inter-species conservation and yet its exact function remains unclear. Even so overexpression of this protein has been reported in lung and colorectal cancers [58, 59]. *ST8SIA2* encodes alpha-2,8-sialyltransferase 8B. Important in this regard is a well-known altered sialic acid processing with secondary to hyper-sialylation of surface glycans [60]. Altered expression of the *ST8SIA2* gene has also been reported in lung and gastric cancers, where its function remains unclear [61, 62]. *DOCK10* is a guanine nucleotide exchange factor (GEF) for Rho GTPases, and its activity is linked to amoeboid invasion and increased metastasis in cutaneous melanoma, breast cancer, and cervical cancer [63–65]. Megakaryocyte-associated tyrosine-protein kinase (*MATK*) is known to be mutated in a small percentage of cutaneous melanoma tumors [66]. The *PSD2* enzyme (phosphatidylethanolamine) has been shown to be elevated in the serum of patients with metastatic lung cancer [67, 68]. Caspase recruitment domain family member 11 (CARD11) is also known to alter NF-kB activity, and while mutations in this gene have been identified in primary vitreoretinal lymphoma, its role in UM has not been evaluated [69]. *EEF1A2* has been identified as an oncogene in breast and ovarian cancers that encodes for elongation factor eEF-1α2 protein, and to our knowledge, its role in UM has not been previously reported [70–72].

Among the top downregulated genes, the enzyme GST alpha 3 (*GSTA3*) plays an important role in steroid biosynthesis, and the downregulation of *GSTA3* has been previously reported in metastatic UM [73]. Both collagen type XI alpha I *(COL11A1)* and synaptoporin *(SYNPR)* are previously shown to be significantly downregulated in monosomy 3 UM [47]. *SYNPR* is an isoform of the major synaptic protein synaptophysin, and its function in cancer remains unclear [74–76]. *COX6A2*, a subunit of Complex IV in the electron transport chain, has been reported to be downregulated in monosomy 3 UM [77–79]. *MSC* is the gene encoding the transcriptional repressor musculin, and in gastric cancer, methylation of the *MSC* promotor is associated with more aggressive tumors [80]. Cardiotrophin-1 (*CTF1)* is a member of the interleukin-6 family of cytokines known to activate STAT3 signaling [81]. Embryonal Fyn-associated substrate (EFS) plays a role in the Src signaling pathway, and hypermethylation of this gene has been shown to be associated with poor

prognosis in UM [82, 83]. *ENPP2* (the autotaxin enzyme) is involved in the synthesis of lysophosphatidic acid and is thought to play a role in cancer metastasis and cancer stem cell function [84]. *AZGP1* encodes zinc-α2-glycoprotein, a tumor suppressor that inhibits TGF-β, and this gene is known to be downregulated in high-risk UM [85, 86]. *IL12RB2* is a subunit of the IL-12 receptor, and while the IL-12 cytokine is known to be differentially expressed in metastatic UM, altered expression of the *IL12RB2* receptor has not been previously reported [87]. Decreased expression of *LIMS2* gene is thought to play a role in tumor metastasis [88]. *MPZ* encodes myelin protein zero, the most abundant protein in myelin, and while mutations and aberrant expression of this gene are implicated in many neuropathic diseases, its role in cancer is not clear [89].

Pathway analyses revealed that differentially expressed genes are enriched in many well-known cancer processes such as cell proliferation, migration, apoptosis, and angiogenesis. Interestingly, chemical synaptic transmission was a top ontology term for the genes identified, and three of the top differentially expressed genes – *HTR2B, RIMS2,* and *SYNPR* – have known synaptic functions. While it has been reported that breast cancer metastases to the brain can develop neuronal-like characteristics, little is known about the role of synaptic proteins in UM metastasis [90].

Interaction network of genes revealed the top players with dense connections to several other genes in the pathway. *ERBB3* is known to be activated by neuregulin-1 and hepatocyte growth factor which provide drug resistance in metastatic UM [91]. Furthermore, ERBB3 signaling is shown to be involved in the survival of melanoma cells after metastasis [92]. This is consistent with its role in tumor development and progression along with other members of the epidermal growth factor receptor family [93]. *PTGS2* is commonly expressed in malignant melanomas and is associated with poor patient survival [94]. In Uveal Melanoma, the expression of Cox-2, a prostaglandin synthase was related to poor prognostic markers including the presence of lymphocytic infiltration, vascular closed loops and the presence of epithelioid cell type in tumors [95]. Network analysis also revealed a total of 20 proteins connected to *GATA4* and *GATA6*, a family of zinc finger proteins. While their role in Uveal Melanoma is yet to be established, *GATA3* is known to interact with hypoxia inducible factor and known to promote the invasiveness of head and neck squamous cell carcinoma [96]. *MYH7B* encodes a heavy chain of myosin II and genome wide association studies have shown that loci containing *MYH7B* gene (20q11.22) is associated with an increased susceptibility to cutaneous melanoma [97].

Conclusion

In conclusion, our analyses confirmed several previously reported genes associated with high-risk UM. We also identified many novel genes that had not been associated with metastatic UM including *KCNQ2, CA8, ST8SIA2, DOCK10, MATK, PSD2, EEF1A2, CARD11, GATA4, MYO7B, C6orf142, LOC100188947, MSC, CTF1, C3orf32, IL12RB2, LIMS2, ZNF835, ERVFRDE1,* and *CLEC11A.* The majority of these genes have known significance in other cancer types, and several have well-known functions related to cancer cell invasiveness and metastasis. This study provides candidates for expanding our understanding of the biology of metastatic UM.

Author Contributions

Conceptualization, AS, LU, KB, SS; Formal analysis, TJL, AV and SK; Methodology, TJL and SK; Project administration, AS; Resources, LU, KB and SS; Supervision, AS; Writing – original draft, TJL, RR and SK; Writing – review & editing, LU, KB, SS and AS.

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