

## Transcriptomic Analysis of Metastatic Uveal Melanoma and Differences in Male and Female Patients

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**Abstract.** *Background/Aim:* Uveal melanoma is an ocular malignancy whose prognosis severely worsens following metastasis. In order to improve the understanding of molecular physiology of metastatic uveal melanoma, we identified genes and pathways implicated in metastatic vs non-metastatic uveal melanoma. *Patients and methods:* A previously published dataset from Gene Expression Omnibus (GEO) was used to identify differentially expressed genes between metastatic and non-metastatic samples as well as to conduct pathway and perturbagen analyses using Gene Set Enrichment Analysis (GSEA), EnrichR, and iLINCS. *Results:* In male metastatic uveal melanoma samples, the gene *LOC401052* is significantly down-regulated and *FHDC1* is significantly up-regulated compared to non-metastatic male samples. In female samples, no significant differently expressed genes were found. Additionally, we identified many significant up-regulated immune response pathways in male metastatic uveal melanoma, including "T cell activation in immune response". In contrast, many top up-regulated female pathways involve iron metabolism, including "heme biosynthetic process". iLINCS perturbagen analysis identified that both male and female samples have similar discordant activity with growth

factor receptors, but only female samples have discordant activity with progesterone receptor agonists. *Conclusion:* Our results from analyzing genes, pathways, and perturbagens demonstrate differences in metastatic processes between sexes.

UM has an incidence rate of 5.1 cases per million people per year (1). UM is found in the choroid (90%), ciliary body (6%), and iris (4%) (2). It is most prevalent in those of fair complexion and light eye color, as well as persons with BRCA-1 mutations (1). The standard treatment for UM is irradiation to local tumor; however, if irradiation therapy is unsuccessful or has severe visual complications, a uvectomy is performed to selectively remove the tumor while attempting to maintain the function of the globe (2, 3).

The prognosis of a localized UM tumor is better than that of metastatic UM which has a 10-year survival rate of 50% (4). This ocular cancer has a high rate of metastasis with the sites of metastasis being primarily the liver (89%), lung (29%), bone (17%), skin (12%), and lymph node (11%) (1). Since metastasis is associated with poor prognosis, we used an omics approach to assess the mechanism of UM metastasis.

Prior work found various genes and driver mutations implicated in UM metastasis. Activating mutations for guanine nucleotide-binding protein alpha Q (GNAQ) and GNA11 genes are linked via initiating cell proliferation (5), while a loss-of-function mutation in BAP1, a gene coding for deubiquitinating enzymes, is correlated with increased risk of metastasis (6). Missense mutations in gene SF3B1, a splicing factor, is also a strong predictor of metastasis (7).

A promising approach to study UM involves bioinformatic analyses of RNAseq datasets. One study assessed microRNA (miRNA) expression in UM with tumor monosomy-3, a predictor of metastasis. The study found miRNAs that were

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*Key Words:* Metastasis, melanoma, uvea, RNA Seq, bioinformatics.



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significantly up-regulated and down-regulated in the event of metastasis (8). A second study used omics data from samples of UM with and without metastasis to analyze copy number variations. This dataset was then used to simulate an evolutionary tree of UM, identify tumor subgroups, response to treatment, and clinical outcomes (9). However, these studies did not investigate differences in RNA expression and associated cellular pathways between samples of metastatic and non-metastatic uveal melanoma.

We sought to extend the knowledge of UM by applying recently developed bioinformatic tools to a previously published UM RNAseq dataset (10). Important elements of our analysis include determining gene and pathway expression differences between metastatic and non-metastatic groups, as well as identifying drugs and compounds that reverse the transcriptome metastatic signature.

## Patients and Methods

**Data re-analysis.** We analyzed a previously published dataset using recently developed bioinformatics tools (11-13). In our analysis we followed recommended best practices for reanalyzing published datasets (14). We downloaded a dataset from a study with a supplementary file consisting of a publicly accessible RNA-seq dataset of UM patients on Gene Expression Omnibus (GEO) (8). The original study evaluated the role epigenetic events have in the development of metastasis UM (8). The dataset consisted of 25 female and 32 male subjects. 33 of the patients had non-metastatic UM and 24 patients had metastatic UM. Six patients' samples did not specify their sex and were excluded from our study. Figure 1 summarizes the workflow used to exclude samples and determine groups for comparison.

Between 2004 and 2010, patients with UM at the Cleveland Clinic Cole Eye Institute who were treated with enucleation had tumor samples snap-frozen and cryopreserved (8). RNA was extracted from the cryopreserved tissue with Trizol Reagent (Invitrogen, Carlsbad, CA, USA), purified, assessed for quality, and scanned on an Illumina BeadArray Reader (Illumina, San Diego, CA, USA) (8). The microarray data then had background subtraction, was normalized, and log transformed using the BeadArray R package v1.0.0 (5). The subsequent RNA microarray dataset was uploaded to Gene Expression Omnibus (GEO) as series GSE44299 (8).

For our re-analysis, we processed the dataset with Geo2R, providing averaged mean expression values for every gene. We compared the two groups with t-tests, identifying differentially expressed genes. Our analysis of this dataset has three components: Gene Set Enrichment Analysis (GSEA), EnrichR, and integrative LINCS (iLINCS) (Figure 2).

**Volcano plot.** The R package ggplot2 was used to create a volcano plot using the DEG gene set to find significant DEGs (Figure 3). The x-axis represents log<sub>2</sub>FC and the y-axis represents the *p*-value using  $-\log_{10}(p\text{-value})$ . We used a log 2-fold change cut off value of  $\pm 1.7$ , depicted by the vertical line. Previous published literature used a cutoff value of 1.0 or greater (15-17). Cut off for *p*-values was set at 0.05 and is represented by the horizontal line. Data points above the horizontal line are significant. Data points lateral to the vertical line have a log 2-fold change value above the threshold.

**Pathway analysis.** Gene Set Enrichment Analysis (GSEA) was used to perform pathway analyses using the full transcriptome. GSEA determined the top up-regulated and down-regulated pathways from an input of ranked genes by adjusted *p*-value and log<sub>2</sub>FC. GSEA's full transcriptome analysis is completed by analyzing the dataset with gene sets instead of singular genes and top pathways are determined by *p*-value and enrichment scores (11). The gene sets were defined by the Gene Ontology pathway package (18).

EnrichR was used to analyze the top 10% up and down-regulated genes. EnrichR is used to generate combined scores to identify pathways that are significantly up or down-regulated from a given lists of DEGs (12).

**Leading edge gene analysis.** GSEA provides a leading-edge (LE) gene analysis in which the genes that are most influential for enrichment of significant pathways are identified (11). LE genes are a subset of genes contributing to the enrichment score in a single pathway gene set. Specifically, three statistics are used to determine the LE subset for a single pathway gene set: Tags, List, and Signal. Tags determine the percentage of genes from a set that contribute to a pathway's enrichment score. From a ranked gene list, List determines the genes that are before (positive), or after (negative) the apex of a pathway's enrichment score. List allows for identification of the location within a gene list where a pathway's enrichment score is established. Signal combines the statistics of both Tags and List to identify leading edge genes (11). Therefore, these LE genes can be interpreted as the core genes within a pathway gene set that account for the pathway gene set's enrichment signal. A gene that is in many of the leading-edge subsets is more likely to be of interest than a gene that is in only a few of the leading-edge subsets. Thus, we analyzed the overlap between multiple leading-edge subsets.

**Signature reversion analysis.** The integrative Library of Integrated Network-based Signatures (LINCS) package was used to find perturbagens that have a discordant signature to that of metastatic activity in UM (13). This analysis compares the L1000 mRNA drug signatures in the iLINCS database to the L1000 mRNA signatures we extracted from the UM DEG list (13).

## Results

**Individual genes.** Significant DEGs were visualized using volcano plots for male and female samples. In male samples, 2 genes were found to be significantly differentially expressed using adjusted *p*-values: LOC401052 is down-regulated and FHDC1 is up-regulated (Figure 3A). Female samples have no significant DEGs by adjusted *p*-Value (Figure 3C). When raw *p*-values are used instead of adjusted ones, additional differentially expressed genes were identified in both male and female samples (Figure 3B and D).

**Pathway analysis.** GSEA for male patients. GSEA in UM male subjects identified 318 significant pathways; 83 were up-regulated and 235 down-regulated (Table I, Table II). In the top 10 up-regulated male pathways, many pathways implicated the immune system, notably B and T cells. The pathways "T cell differentiation in immune response", "T cell mediated immunity", "B cell receptor signaling

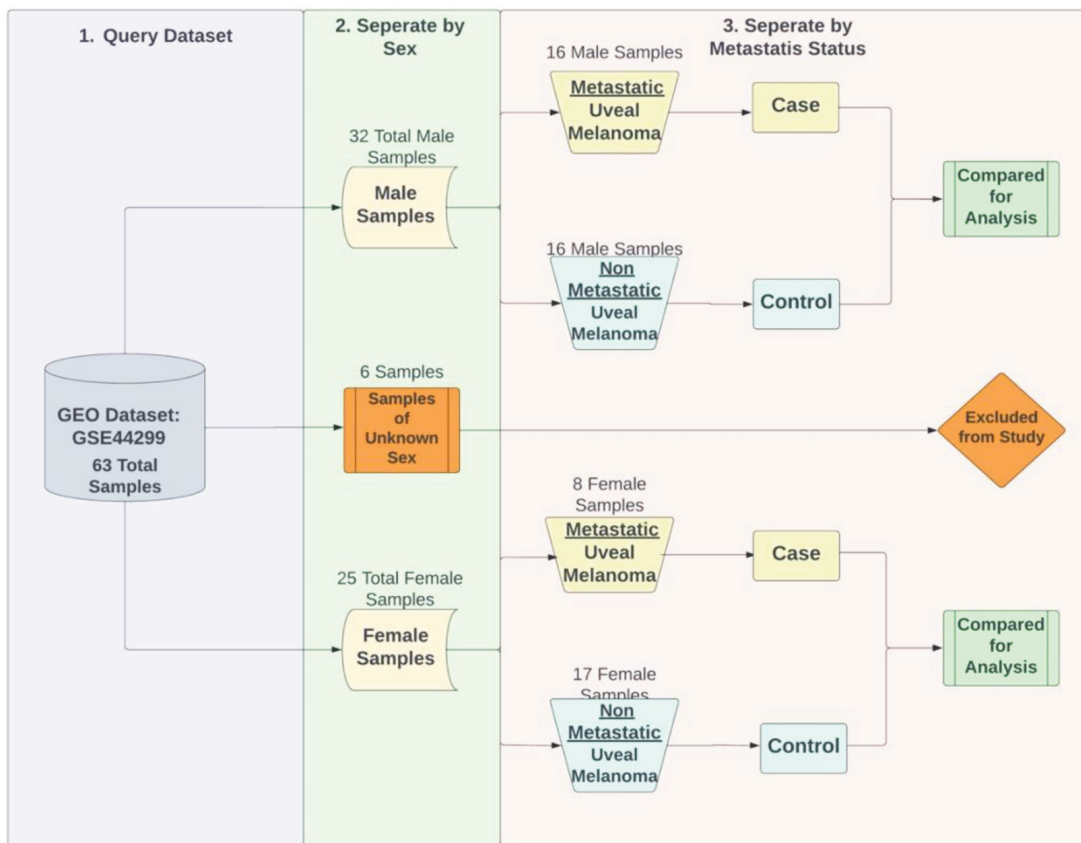


Figure 1. Overall workflow. In step 1, the GEO Dataset GSE44299 was selected. In step 2, male and female samples were separated while samples of unknown sex were excluded. In step 3, within each of the sex-based groups, samples were separated by metastatic activity and compared.

pathway”, “B cell homeostasis”, and “T cell activation in immune response” were significantly up-regulated (Table I). Top down-regulated pathways in male samples involved lipid function, such as “plasma lipoprotein organization”, “protein-lipid complex subunit organization”, “triglyceride metabolic process”, and “neutral lipid metabolic process” (Table II).

*GSEA for female patients.* GSEA in UM female subjects identified a total of 172 significant pathways, with 95 up-regulated and 77 down-regulated. Of the top 10 up-regulated pathways from this list, five are implicated in cellular iron metabolism. The pathways “heme biosynthetic process”, “porphyrin compound biosynthetic process”, “heme metabolic process”, and “porphyrin compound metabolic process” were up-regulated (Table III). The top 10 down-regulated pathways (Table IV) show that pathways positively regulating kinase activity are significantly down-regulated, such as the “positive regulation of P13K activity” and “positive regulation of lipid kinase activity” pathways.

*EnrichR for male patients.* The EnrichR analysis of male subjects identified a total of 968 pathways; 435 were up-regulated and 533 down-regulated. Of the top 10 pathways, two were involved with Notch signaling, *i.e.*, “positive regulation of Notch signaling pathway”, and “regulation of Notch signaling pathway” (Table V, Table VI). The top down-regulated pathway, “negative regulation of serine/threonine kinase activity”, was also found as a top down-regulated pathway in GSEA.

*EnrichR for female patients.* EnrichR analysis of female subjects identified 923 significant pathways; 458 were up-regulated and 465 down-regulated (Table VII, Table VIII). Top pathways in these groups involve cellular processes.

*Leading edge gene analysis.* Leading edge gene analysis of female samples and male samples using GSEA (Figure 4) found that the genes ATM and P2RX7 were present in both the male and female top up-regulated LE genes lists. No similar genes were found in top down-regulated LE genes in the two

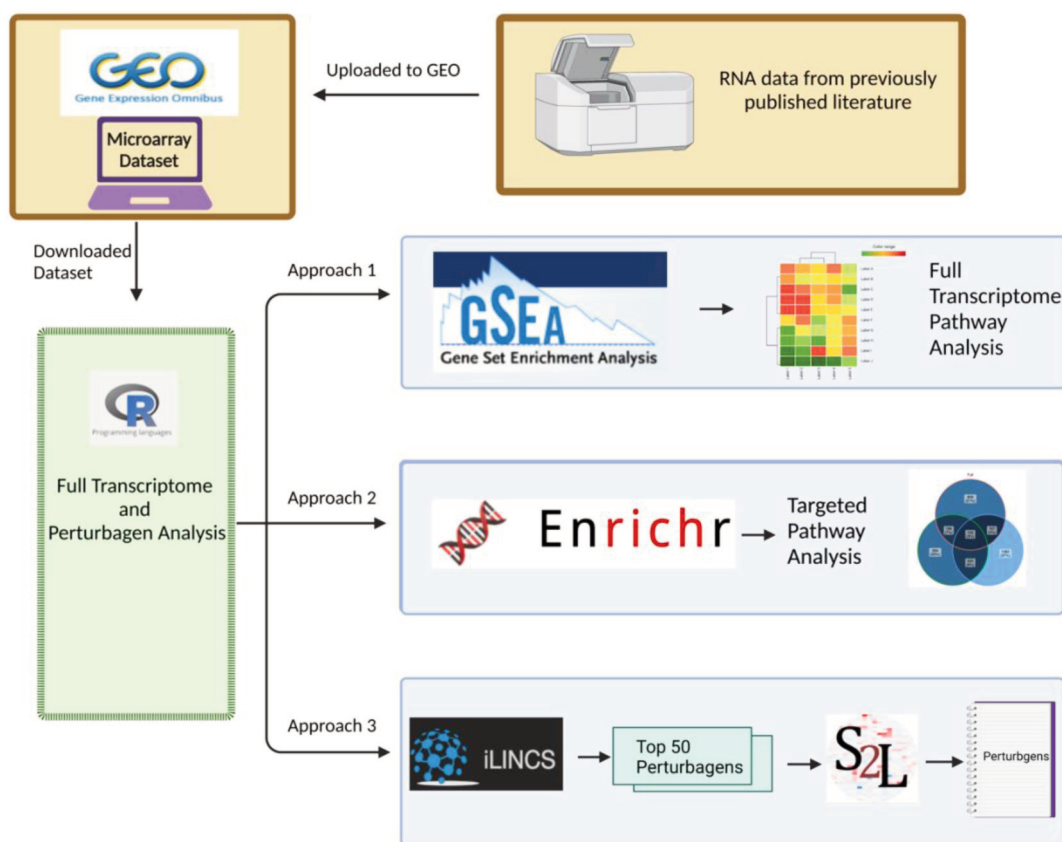


Figure 2. Workflow of analysis. RNA data from published literature were found on the Geo Expression Omnibus (GEO). The samples were downloaded and analyzed with Gene Set Enrichment Analysis (GSEA), EnrichR, and iLINCS.

lists. The genes APOE and RHOE were the top up-regulated LE genes in female samples but were also in the top down-regulated LE genes in male samples. Additionally, the gene LYN is a top up-regulated LE gene in male samples and a top down-regulated LE gene in female samples (Figure 4).

**Perturbagen analysis.** Perturbagen analysis using iLINCS found chemical compounds producing a discordant signature to that of metastatic activity in UM in female and male samples (Table IX, Table X). When analyzing the female dataset, the FDA approved drug Levonorgestrel, a progesterone agonist, had a discordance score of  $-0.45$  (Table IX). Analysis of the male dataset found that Rosuvastatin, an HMGCR inhibitor used as a cholesterol medication, had a discordant score of  $-0.73$  (Table X). Further analysis of discordant perturbagens for female metastatic UM found nine discordant perturbagens with progesterone receptor agonists as a mechanism of action (MOA) (Table XI). In male samples, 16 discordant perturbagens had the MOA of P13K inhibition (Table XII). Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGFR)

were the top 10 most common MOAs in both male and female discordant perturbagens (Table XI, Table XII).

**Common perturbagen chemical moieties.** Structural analysis was performed manually by reviewing the top 100 perturbagens in the female and male datasets for common structural motifs. A steroid-like backbone was observed in 26% of the structures (Figure 5A). No other substructure or chemical motif accounted for more than 5% of the list. In the male list, in a similar fashion to the females, a steroid-like backbone was the most prevalent, but only made up 8% of the library. None of the steroid-like perturbagens were common between the datasets. Although the steroid substructure makes up the most significant portion of each list relative to other chemical classes, no individual steroid-like perturbagen was found on both lists. Smaller common fragments were identified in both lists (Figure 5B and C).

## Discussion

With metastatic activity in UM being a leading cause of mortality from the disease, understanding the molecular

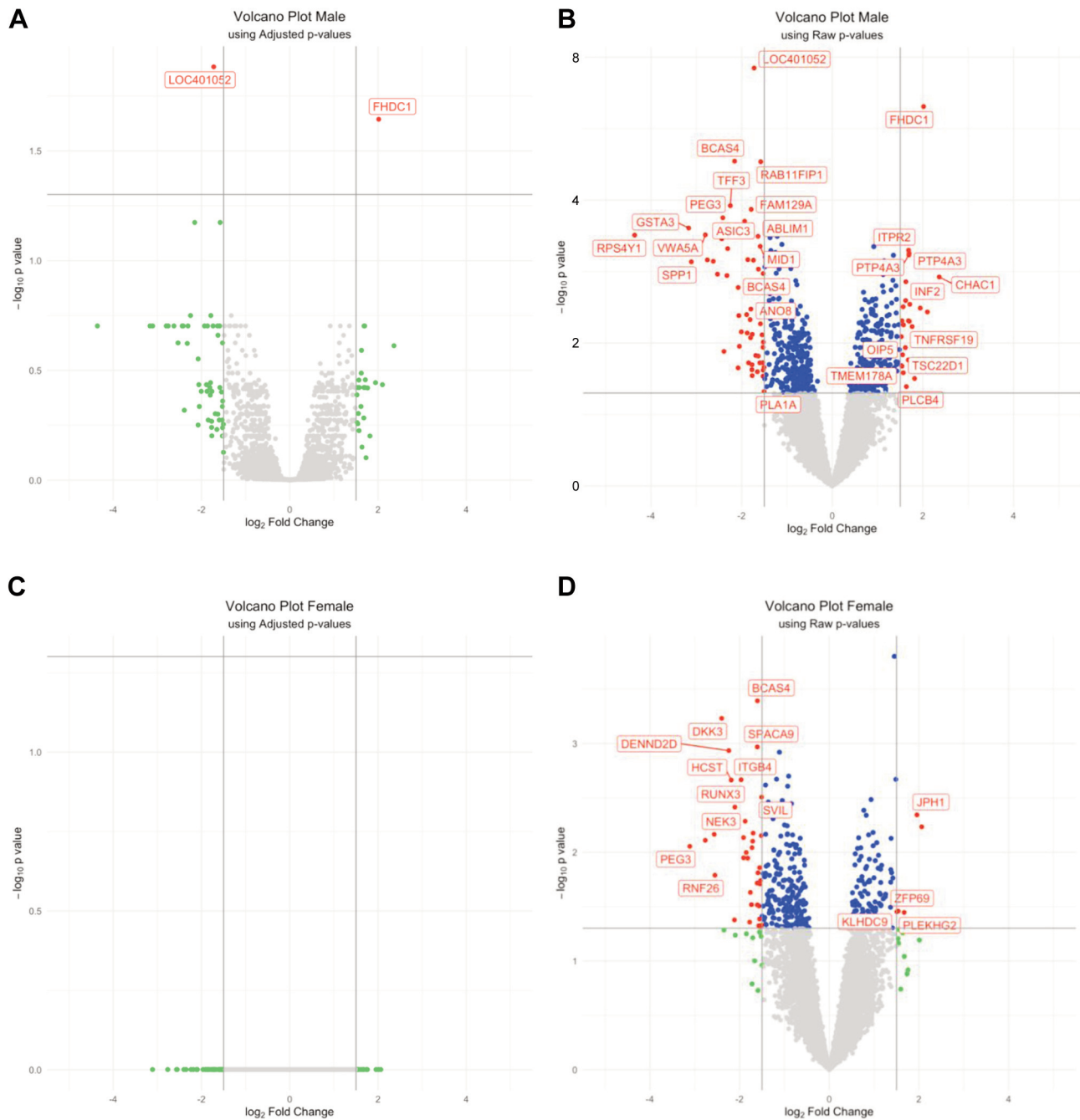


Figure 3. Volcano plot of differentially expressed genes (DEGs). (A) depicts a volcano plot of log 2-fold change and adjusted p-value scores of male uveal melanoma (UM) samples. Blue dots represent significant down-regulated genes and red dots represent significant up-regulated genes. *FHDC1* is significantly up-regulated and *LOC401052* is significantly down-regulated. (B) depicts a volcano plot of log 2-fold change and raw p-value scores of male UM samples. (C) depicts the log 2-fold change and adjusted p-value scores of female UM samples. (D) depicts the log 2-fold change and raw p-value scores of female UM samples.

physiology of metastasis can serve to improve patient outcomes (4). While current knowledge has uncovered specific genes implicated in metastatic activity (5-7, 19, 20), pathway analysis and cellular mechanisms implicated in

metastasis may provide further insights. We investigated the metastatic *versus* non-metastatic uveal melanoma transcriptional profiles using current bioinformatic approaches to identify significant pathways, genes, and perturbagens. We

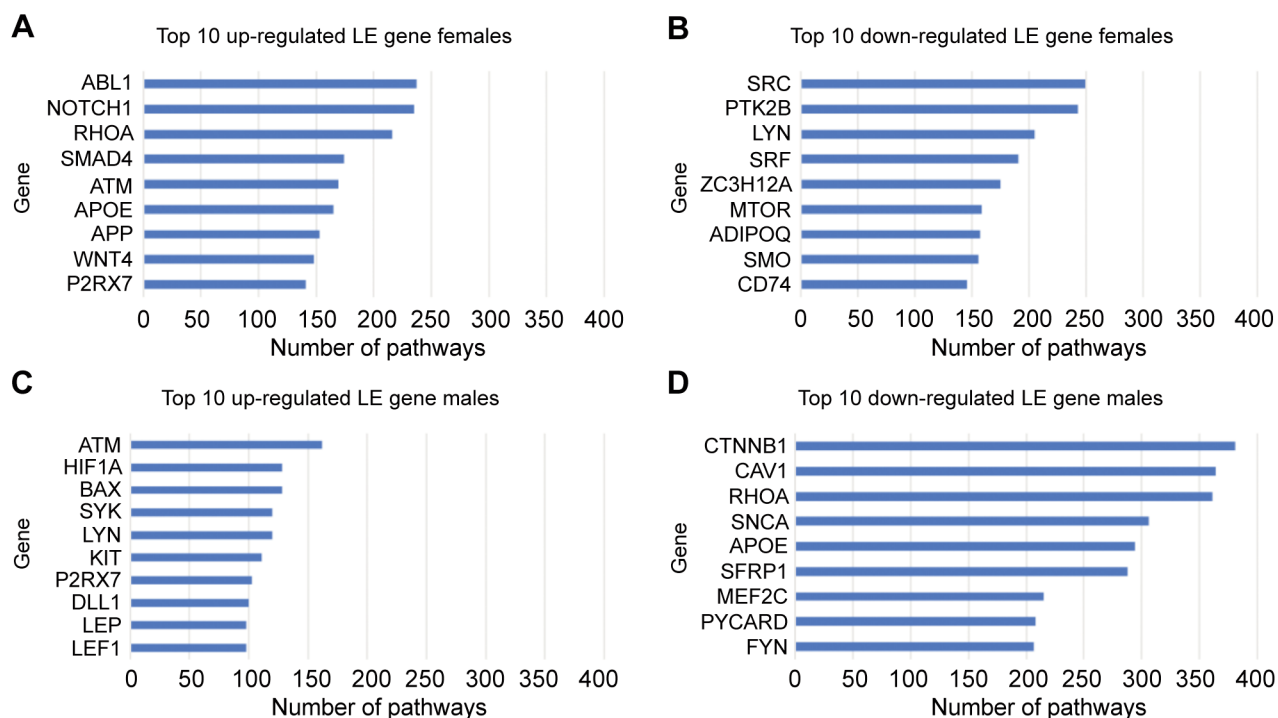


Figure 4. Leading edge gene analysis for female and male samples: Identification of the ten most common differentially expressed genes in pathways of interest in metastatic uveal melanoma in female and male samples. (A) depicts the top 10 up-regulated LE genes in female samples. (B) depicts the top 10 down-regulated LE genes in female samples. (C) depicts the top 10 up-regulated LE genes in male samples. (D) depicts the top 10 down-regulated LE genes in male samples. The X axis represents the number of pathways that are influenced by the gene on the Y axis. LE, Leading edge.

identified genes and pathways that have been previously implicated in metastatic UM and metastatic cancer, as well as novel pathways and genes.

The original report analyzing this dataset did not assess the effect of sex. Since sexual dimorphism is an impactful factor that may impact pathogenesis of UM, we analyzed the dataset as two separate cohorts. We found that the expression profiles, pathways, leading edge genes, and perturbagens differed by sex. These findings suggest at minimum that examination of uveal melanoma should be done separately in male *versus* female UM.

*Significant pathways and leading-edge genes in females.* Using GSEA, the top 10 up-regulated pathways implicated in metastatic activity in female uveal melanoma samples were identified by adjusted *p*-value (Table I). A common theme of up-regulated pathways is that many involve hemoglobin. These findings are congruent to current literature, as previously an *in vitro* study found that Heme Oxygenase-1 overexpression is associated with progression of UM (21). A second intriguing pathway is the “placenta blood vessel development” as maturation of blood vessels in UM is correlated to increased cell proliferation and eventual metastasis (22). These pathways suggest that metastatic activity in UM in females may rely on

increased vascularization of the tumor. A down-regulated pathway in the female cohort, “positive regulation of P13K regulation”, was an interesting finding as many cancers are associated with overactivation of this growth regulating signaling pathway (Table II) (23).

Previously, a multitude of genes were implicated in the growth and invasion of uveal melanoma. Our leading-edge gene analysis conducted with GSEA using female uveal melanoma samples identified genes previously found in UM, including NOTCH1, and SMAD4 [up-regulated (24-26)], and PTK2B and MTOR [down-regulated (27-30)] (Figure 4A and B).

*Significant pathways and leading-edge genes in males.* The top 10 up-regulated pathways in male samples by adjusted *p*-Value have a common theme (Table III). Immune pathways, such as “B Cell Differentiation”, “Immune Effector Process”, “Lymphocyte Differentiation”, “Mononuclear Cell Differentiation”, “Lymphocyte Activation in Immune Response”, “B Cell Activation”, and “Leukocyte Differentiation” are up-regulated in metastatic UM. These pathways support previous findings of UM metastasis being caused in part by immune evasion (31).

The top down-regulated pathway “regulation of adenylate cyclase activity” suggests that metastasis of uveal melanoma

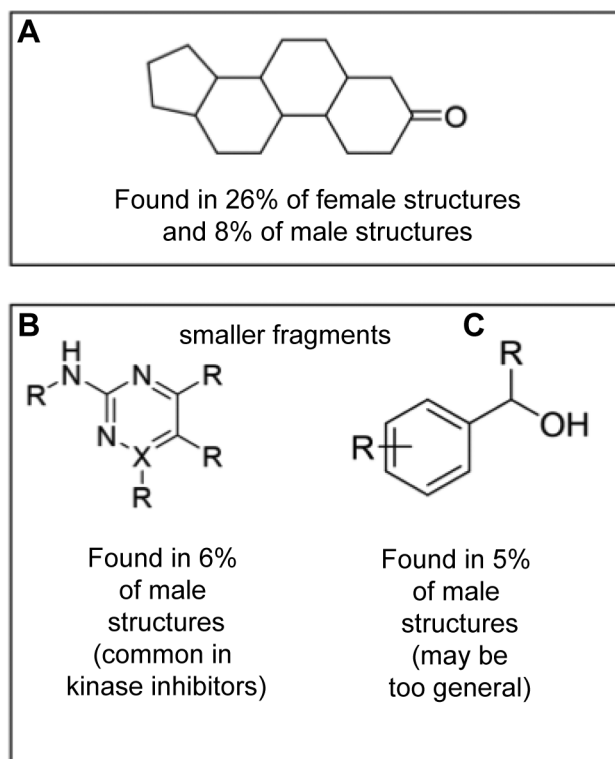


Figure 5. Common substructures observed in discordant perturbagens. The structures of discordant male and females perturbagens were assessed to identify common structures. (A) depicts a structure found in 26% of female samples and 8% of male samples. (B) shows two other smaller common substructures.

Table I. GSEA analysis of up-regulated pathways in male samples.

Pathway	Rank female	p-Adj	p-Value
Positive regulation NOTCH Signaling	1	0.05	<0.01
B Cell differentiation	2	0.05	<0.01
Regulation of NOTCH signaling pathway	3	0.07	<0.01
Immune effector process	4	0.08	<0.01
Lymphocyte differentiation	5	0.09	<0.01
Mononuclear cell differentiation	6	0.12	<0.01
Golgi apparatus subcompartment	7	0.12	<0.01
Lymphocyte activation in immune response	8	0.16	<0.01
B Cell activation	9	0.17	<0.01
Leukocyte differentiation	10	0.18	<0.01

p-adj, Adjusted p-Value; n/a, not available.

is related to increased adenylate cyclase activity. Similarly, increased activity of adenylate cyclase associated protein-1 (CAP1) is associated with metastasis of lung cancer (32). Down-regulation of the “negative regulation of canonical

Table II. GSEA analysis of down-regulated pathways in male samples.

Pathway	Rank female	p-Adj	p-Value
Negative regulation of STK activity	1	0.01	<0.01
Cytosolic ribosome	2	0.01	<0.01
Ribosomal subunit	3	0.01	<0.01
Cytoplasmic translation	4	0.01	<0.01
Neutral lipid metabolic process	5	0.01	<0.01
Negative regulation of canonical WNT pathway	6	0.02	<0.01
Negative regulation of cell proliferation	7	0.05	<0.01
Small ribosomal subunit	8	0.05	<0.01
Cytosolic small ribosomal subunit	9	0.05	<0.01
Acylglycerol metabolic process	10	0.05	<0.01

p-adj, adjusted p-Value; STK, serine/threonine kinase.

Table III. GSEA analysis of up-regulated pathways in female samples.

Pathway	Rank female	p-Adj	p-Value
Placenta blood vessel development	1	0.70	<0.01
Heme biosynthetic process	2	0.70	<0.01
Porphyrin compound biosynthetic process	3	0.70	<0.01
Tetrapyrrole biosynthetic process	4	0.70	<0.01
Heme metabolic process	5	0.70	<0.01
Porphyrin compound metabolic process	6	0.70	<0.01
Adherens junction	7	0.70	<0.01
Retromer complex	8	0.80	<0.01
Alternative mRNA splicing	9	0.80	<0.01
Regulation of autophagy of mitochondrion	10	0.80	<0.01

p-adj, adjusted p-Value; mRNA, messenger ribosomal nucleic acid.

WNT pathway” in the metastasis of uveal melanoma is supported by previous findings that the WNT pathway supports tumorigenesis and metastasis (33).

Leading edge gene analysis of male samples found genes previously identified in the progression of uveal melanoma (Figure 4C): ATM, BAX, KIT, and P2RX7 (34-36), while one down-regulated gene found in samples exhibiting metastatic activity was identified: FYN (37) (Figure 4D). A notable finding was the down-regulation of the CAV1 gene. Previously, researchers found metastatic disease to be associated with higher Cav-1 expression, suggesting that further investigations need to be conducted on this incongruence (38).

*iLINCS connectivity and perturbagen analysis.* We found VEGFR and EGFR inhibitors are the two top discordant mechanism of actions found in both male and female samples (Table XI, Table XII). Current treatment for UM

Table IV. GSEA analysis of down-regulated pathways in female samples.

Pathway	Rank female	p-Adj	p-Value
Membrane microdomain	1	0.70	<0.01
Membrane raft	2	0.70	<0.01
Positive regulation lipid kinase	3	0.70	<0.01
Positive regulation P13K	4	0.70	<0.01
Cell surface	5	0.80	<0.01
Positive regulation of NF-kappaB	6	0.80	<0.01
Positive regulation of intracellular transport	7	0.80	<0.01
Plasma membrane signaling receptor	8	0.80	<0.01
Circadian regulation of gene expression	9	0.80	<0.01
Peripheral nervous system development	10	0.80	<0.01

p-adj, adjusted p-Value; P13K, phosphoinositide 3-kinase; NF-kappaB, nuclear factor kappa-light-chain-enhancer of activated B cells.

Table V. EnrichR analysis of up-regulated pathways in male samples.

Pathway	Rank male	p-Adj	p-Value
Positive regulation of Notch signaling pathway	1	0.07	<0.01
IRE1-mediated unfolded protein response	2	0.07	<0.01
Positive regulation of signal transduction	3	0.07	<0.01
Regulation of Notch signaling pathway	4	0.08	<0.01
Negative regulation of cell volume	5	0.27	<0.01
Renal tubule development	6	0.27	<0.01
Positive regulation of endocytosis	7	0.27	<0.01
Retinal ganglion cell axon guidance	8	0.27	<0.01
Actin filament-based transport	9	0.27	<0.01
Regulation of cell development	10	0.27	<0.01

p-adj, adjusted p-Value.

includes the VEGF inhibitor Cabozantinib (39). Additionally, EGFR inhibitors are being investigated for uveal melanoma *in vitro* (40, 41).

**Identification of sex differences.** We separated the dataset by sex into two cohorts as current literature supports sex differences in UM (42, 43). There was no overlap of significant GSEA or EnrichR pathways between the two sexes. However, the leading edge genes ATM and P2RX7 had increased expression in both sexes. This finding suggests that these genes have a strong role in tumor progression regardless of sex. Previously, researchers found that loss of ATM increased the risk of metastasis (34), while increased expression of the P2RX7 gene was associated with increased tumorigenesis (44).

Three leading edge genes had divergent changes between sexes. The gene LYN, whose down-regulation is implicated in

Table VI. EnrichR analysis of down-regulated pathways in male samples.

Pathway	Rank male	p-Adj	p-Value
Negative regulation of serine/threonine kinase	1	0.02	<0.01
Negative regulation of cell population proliferation	2	0.02	<0.01
Skeletal muscle organ development	3	0.03	<0.01
Negative regulation of signal transduction	4	0.05	<0.01
Negative regulation of MAPK cascade	5	0.05	<0.01
Cellular protein metabolic process	6	0.07	<0.01
Negative regulation of cellular process	7	0.07	<0.01
Organonitrogen compound biosynthetic process	8	0.07	<0.01
Endothelial tube morphogenesis	9	0.09	<0.01
High-density lipoprotein particle remodeling	10	0.09	<0.01

p-adj, adjusted p-Value; MAPK, mitogen-activated protein kinase.

Table VII. EnrichR analysis of up-regulated pathways in female samples.

Pathway	Rank female	p-Adj	p-Value
Intracellular membrane-bounded organelle	1	<0.01	<0.01
Cell nucleus	2	0.02	<0.01
Spliceosomal tri-snRNP complex	3	0.03	<0.01
U4/U6 x U5 tri-snRNP complex	4	0.03	<0.01
Late endosome membrane	5	0.03	<0.01
Lytic vacuole membrane	6	0.03	<0.01
Cell-substrate junction	7	0.03	<0.01
Lysosome	8	0.03	<0.01
Focal adhesion	9	0.03	<0.01
Bounding membrane of organelle	10	0.03	<0.01

p-adj, adjusted p-Value; snRNP, small nuclear ribonucleoprotein.

aggressive breast cancer (45), was a top 10 down-regulated LE gene in females, but a top 10 up-regulated LE gene in males. In male samples, the gene RHOA is a top down-regulated LE gene while in female samples RHOA is a top up-regulated LE gene. RHOA is implicated in breast and ovarian and its expression is associated with increased progression due to increased cancer motility and invasiveness (46, 47). Another disagreement in the findings is the APOE gene, which was up-regulated in female samples and down-regulated in male samples. APOE4 is associated with a favorable outcome, while APOE2 with the unfavorable outcomes of progression and metastasis (48). Top discordant mechanisms of actions found only in females include estrogen and progesterone receptor agonists [Table X]. Additionally, we found a steroid-like backbone to be a common recurring chemical moiety in discordant female perturbagens.



Table VIII. *EnrichR analysis of down-regulated pathways in female samples.*

Pathway	Rank female	p-Adj	p-Value
RNA binding	1	<0.01	<0.01
Lysosome	2	<0.01	<0.01
Intracellular non-membrane-bounded organelle	3	<0.01	<0.01
Intracellular membrane-bounded organelle	4	<0.01	<0.01
Nucleus	5	<0.01	<0.01
Lysosomal membrane	6	<0.01	<0.01
Nuclear lumen	7	<0.01	<0.01
Nucleolus	8	<0.01	<0.01
Lytic vacuole membrane	9	<0.01	<0.01
Endosome membrane	10	0.01	<0.01

p-adj, adjusted p-Value.

Table IX. *Discordant perturbagens for female metastatic uveal melanoma.*

Perturbagen	Discordant score	Mechanism of action
MLS002460474	-0.60	Unknown
GF-109293	-0.56	CDK inhibitor
CHEML1269202	-0.54	BCL inhibitor
BRD-K80230516	-0.53	Unknown
Benzylimidazole	-0.51	Thromboxane inhibitor
Tyrphostin AG 825	-0.47	EGFR inhibitor
Broad-Sai-045	-0.46	Unknown
Purmorphamine	-0.46	Smoothened receptor agonist
Levonorgestrel	-0.45	Progesterone agonist
CHEMBL2136735	-0.45	FGFR inhibitor

CDK, Cyclin-dependent kinases; BCL, B-cell leukemia/lymphoma 2 protein; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor 1.

Table X. *Discordant perturbagens for male metastatic uveal melanoma.*

Perturbagen	Discordant score	Mechanism of action
Rosuvastatin	-0.73	HMGCR inhibitor
Ammonium Glycyrrhizinate	-0.72	Unknown
AZD4547	-0.70	Unknown
Elvitegravir	-0.69	HIV integrase inhibitor
10-DEBC	-0.69	AKT inhibitor
PF-562271	-0.68	Focal adhesion kinase inhibitor
Lomitapide	-0.68	Triglyceride transfer inhibitor
Lonidamine	-0.68	Glucokinase inhibitor
PF-04691502	-0.68	Serine/threonine kinase inhibitor
Voglibose	-0.68	alpha-glucosidase inhibitor

HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; HIV, human immunodeficiency virus; AKT, protein kinase B.

Table XI. *Top 10 discordant perturbagen MOA for female metastatic uveal melanoma.*

Mechanism of action	Number of perturbagens associated with MOA
Progesterone receptor agonist	9
CDK inhibitor	5
EGFR inhibitor	4
VEGFR inhibitor	4
Estrogen receptor agonist	3
PKC inhibitor	3
Androgen receptor modulator	2
Aromatase inhibitor	2
BCL inhibitor	2
Cytochrome P450 inhibitor	2

MOA, Mechanism of action; CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; VEGFR, vascular endothelial growth factor; PKC, protein kinase C; BCL, B-cell leukemia/lymphoma 2 protein.

Table XII. *Top 10 discordant perturbagen MOA for male metastatic uveal melanoma.*

Mechanism of action	Number of perturbagens associated with MOA
PI3K inhibitor	16
ATPase inhibitor	13
Adrenergic receptor antagonist	11
VEGFR inhibitor	11
EGFR inhibitor	10
HDAC inhibitor	10
MTOR inhibitor	10
PDGFR tyrosine kinase receptor inhibitor	9
Calcium channel blocker	8
Cyclooxygenase inhibitor	8

MOA, Mechanism of action; PI3K, phosphoinositide 3-kinase; VEGFR, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; MTOR, mechanistic target of rapamycin; PDGFR, platelet-derived growth factor receptor.

## Conclusion

By combining GSEA, EnrichR, and iLINCS, we were able to identify new information from a previously published dataset. Separating samples by sex allowed us to obtain better insight on the differences in processes between the two cohorts. Further studies investigating functional proteomic data of metastatic UM between sex and replication of our findings may provide new leads for the field.

## Conflicts of Interest

None.

## Authors' Contributions

SD: Conceptualization, data curation, original investigation, statistical analysis, manuscript writing. AH: data curation, statistical analysis, manuscript writing. HE: data curation, statistical analysis, manuscript writing. XZ: data curation, statistical analysis. AI: data curation, statistical analysis. ES: Conceptualization statistical analysis. IS: analysis. JML: analysis. DG: analysis, conceptualization, oversight. JM: analysis, conceptualization, oversight. RM: conceptualization, oversight, reviewing and editing.

## Acknowledgements

This work was supported by National Institute of Health (NIMH) MH 107487, MH 121102, and AG057598 (REM).

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Received February 17, 2024

Revised March 20, 2024

Accepted April 2, 2024