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Nomogram of uveal melanoma as prediction model of metastasis risk

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ABSTRACT

Background: Since the poor prognosis of uveal melanoma with distant metastasis, we intended to screen out possible biomarkers for uveal melanoma metastasis risk and establish a nomogram model for predicting the risk of uveal melanoma (UVM) metastasis.
Methods: Two datasets of UVM (GSE84976, GSE22138) were selected. Data was analyzed by R language, CTD database and GEPIA.
Results: The co-upregulated genes of two datasets, HTR2B, CHAC1, AHNAK2, and PTP4A3 were identified using a Venn diagram. These biomarkers are combined with clinical characteristics, and Lasso regression was conducted to filter the metastasis-related biomarkers. HTR2B, CHAC1, AHNAK2, PTP4A3, tumor thickness, and retinal detachment (RD) were selected to establish the nomogram.
Conclusion: Our study provides a comprehensive predictive model and personalized risk estimation tool for assessment of 3-year metastasis risk of UVM with a better accuracy.

1. Introduction

Uveal melanoma (UVM) is the most common intraocular cancer that deriving from melanocytes in the uvea [1], with an incidence rate of 5–7 cases per million people per year [2]. Currently, there are several treatments for uveal melanoma, including surgery, radiation therapy, laser and photodynamic therapy, chemotherapy, and immunotherapy [3]. However, little progress in terms of

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Abbreviations

UVM	Uveal melanoma
HTR2B	Serotonin receptor
CHAC1	Cation transport regulator-like protein 1
AHNAK2	AHNAK nucleoprotein 2
PTP4A3	Phosphatase type IV A member 3
GNAQ	Guanine nucleotide-binding protein alpha Q
DEGs	Differentially expressed genes
EMT	Epithelial-mesenchymal transition
GTPase	Guanosine triphosphate
HGF	Hepatocyte growth factor
MET	Mesenchymal-epithelial transition factor

survival rate has been made in the past 25 years [4–6]. Actually, though successfully treating the primary tumor at early stage, many patients will still develop into metastatic disease [7]. Once distant metastasis occurs, the mortality rate can be up to 50% [4,8]. Therefore, the identification of novel biomarkers for the metastasis risk is urgent.

The risk of UVM metastasis can be decided by molecular biomarkers. Cancer cells gain the ability of metastases by gene mutations and abnormal expression [9]. Hundreds of genes are involved in the cancer metastasis, including TP53, CDKNA2, PTEN, PIK3CA [10]. There is heterogeneity. In uveal melanoma, several genes have been reported as the potential biomarkers for metastasis, such as PBRM1, EZH2 mutations [11], high protein tyrosine phosphatase type IV A member 3 (PTP4A3) expression [12], and metabolism related genes (carbonic anhydrase 12, acyl-CoA synthetase long-chain family member 3, and synaptojanin 2) [13]. Therefore, the biomarkers related to metastasis could suggest the risk of UVM metastasis [14].

Nomogram is a predictive model widely used in cancer prognosis [15]. In addition to clinical factors, we wonder if key genes could be selected as biomarkers for tumor metastasis, and improve the efficacy and accuracy of UVM nomogram.

In our study, we identified four genes that were possibly related to the pathogenesis of UVM metastasis. Three of these four genes, including HTR2B, CHAC1, AHNAK2, and PTP4A3, were selected as possible biomarkers for prediction of UVM metastasis. Nomogram based on three identified genes as well as two clinical characteristics were established. Four biomarkers could bring more net benefits and enhance the efficacy of the model. Our study provides a reliable predictive model and personalized risk estimation tool for prognosis assessment with a better accuracy.

2. Method

2.1. Data processing

We searched GEO database (https://www.ncbi.nlm.nih.gov/gds) and CTD (http://ctdbase.org/) which are public databases of chips and microarrays. We searched keywords including “uveal melanoma” in the two databases above.

GSE22138, GSE84976 were downloaded from GEO database, analyzed by R programming language. All the data were normalized first, then the differentially expressed genes (DEGs) were calculated by limma algorithm packages. The log fold change cutoff and adjusted p value or p value were set as 1.5 and 0.05 respectively. The volcano plot was drawn by R programming language. The co-DEGs were identified by venn diagram, an online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) which can calculate the intersections of gene lists.

The CTD database (Comparative Toxicogenomics Database) supply the relationship between genes and diseases (http://ctdbase.org/). Search the target genes, rank the diseases involved by inference score.

GEPIA tool (Gene Expression Profiling Interactive Analysis) (http://gepia.cancer-pku.cn/) were online tools used for the search the gene expression in various and survival analysis.

Lasso regression and logistic regression are analyzed by R programming language. Nomogram was conducted by rms R package. Forest plot was conducted by forest plot R package. Calibration curve, decision curve and ROC curve were conducted by R programming language.

2.2. Identification of protein-protein interaction networks

The protein –protein interaction networks were calculated on online tool ，String (https://www.string-db.org/). Downloaded the network information and imported it to cytoscape (v3.7.1), calculated the most important networks by MCODE and cytohubba algorithm. Adjust the color of each node according to the interaction quantities.

2.3. Functional enrichment analysis

GO enrichment analysis of DEGs was calculated on the online tool DAVID (https://david.ncifcrf.gov/home.jsp, version 6.8). The

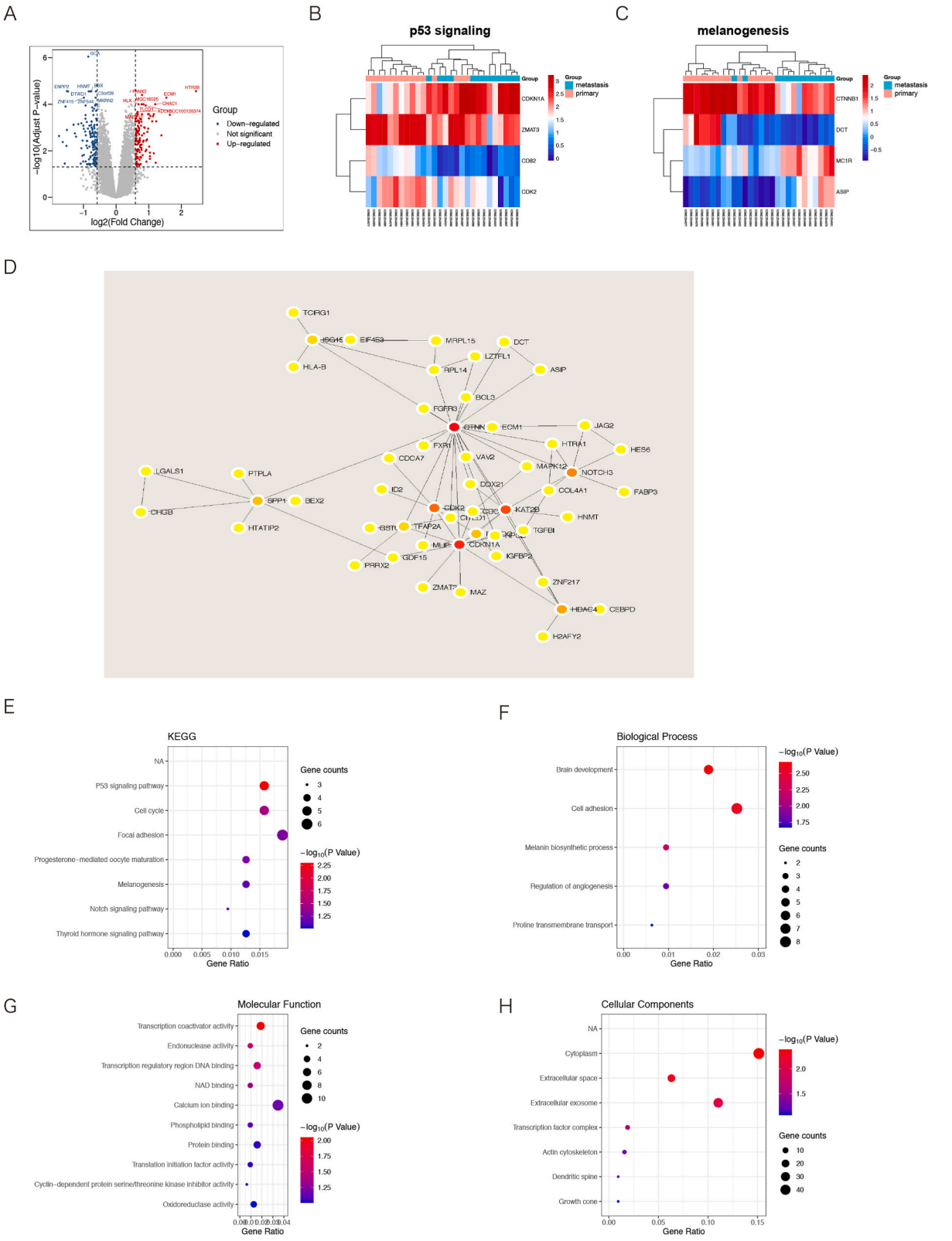

Fig. 1. DEGs, PPI network and GO analysis of a dataset of uveal melanoma, GSE84976. A. Volcano plot shows the up-regulated and down-regulated genes in GSE84976. B. Heatmaps of P53 signaling and melanogenesis pathways. C. Protein-protein interaction (PPI) networks, which shows the interaction and sub-network between DEGs. E-H. KEGG pathway (E) and GO analysis (F–H) showed possible pathways in uvm.

bubble maps were drawn using Hmisc and ggplot2 via the R programming language.

3. Results

3.1. Identification of DEGs , PPI network and functional enrichment of DEGs

We searched the GEO database for information on both UVM, and then chose datasets GSE84976, GSE22138. Demographic variable data of GSE22138 (12) and GSE84976 [16] have been detailed presented in the previous studies. We then divided the patients into two groups based on the presence of metastasis and were able to identify 264 DEGs in GSE84976, a dataset on UVM. This included 120 up-regulated genes and 144 down-regulated genes (Fig. 1A). To further explore the function of DEGs, we conducted gene ontology (GO) analysis on each dataset. P53 signaling and melanogenesis pathways are involved in the development of uveal melanoma, and the resulting heatmap showed the gene expression patterns in the two pathways (Fig. 1B and C). We analyzed the functional protein association network using STRING and visualized the results with Cytoscape software (Fig. 1D); the results showed the interactions and sub-networks between DEGs. KEGG pathway (Fig. 1E) analysis demonstrated that UVM-DEGs were mainly enriched by p53 signaling, and cell cycle. Additionally, GO analysis (Fig. 1F, G, 1H) showed that the regulation of melanin biosynthetic process, angiogenesis, and cell adhesion were associated with the process of UVM. GSE22138 has 12 up-regulated genes and 8 down-regulated genes, as is shown in the volcano plot (Fig. 2A) and the heatmap (Fig. 2B).

To explore the common up-deregulated genes in both datasets of UVM, we analyzed the co-DEGs of two datasets using a Venn diagram (Fig. 2C). There were four overlapped genes, including HTR2B, CHAC1, AHNAK2, and PTP4A3. In addition, we noticed that HTR2B is ranked first by logFC in both datasets.

3.2. CTD database searching

To further explore the relationship between these four genes and the diseases, we use a CTD database, which helps define the genes

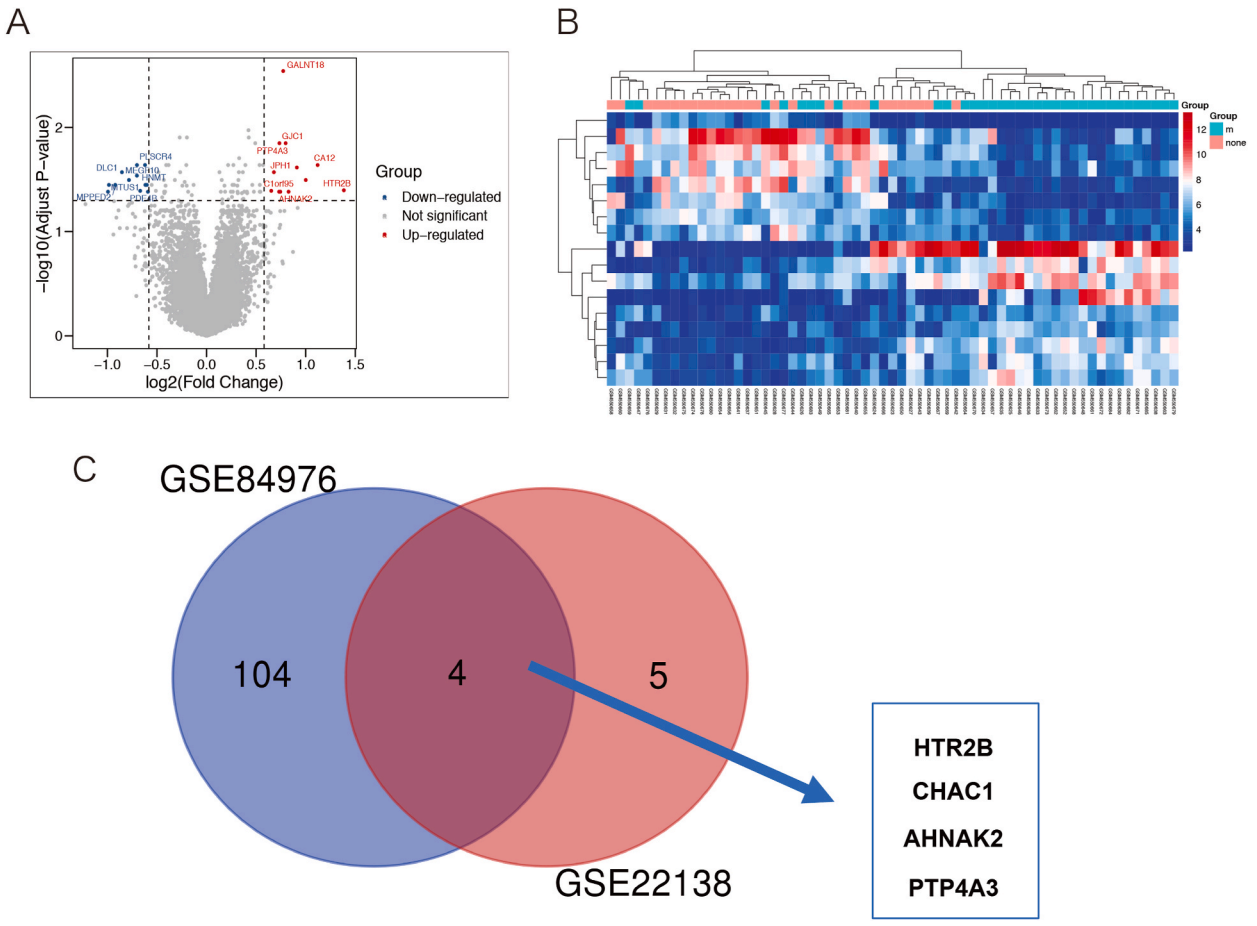

Fig. 2. A. Volcano plots show the up-regulated and down-regulated genes in GSE22138. B. Heatmaps of GSE22138. C. Identification of co-DEGs. Four co-DEGs of two datasets were identified by Venn diagram, including HTR2B, CHAC1, AHNAK2, PTP4A3.

that particular diseases target. We focused specifically on cancer, metastases, and ocular diseases. As shown in Fig. 3, all four genes are related to neoplasms, neoplasm invasiveness, and uveal or eye diseases. (Fig. 3A, B, C, D). These results further confirmed the crucial role of all four in UVM.

3.3. The important roles of HTR2B, CHAC1, AHNAK2, PTP4A3 in UVM

We conducted survival analysis using GEPIA to further explore the functions of the four previously mentioned co-expressed genes in UVM. The Kaplan-Meier plotter suggested that patients with higher expressions of HTR2B ($p = 0.0012$) have a lower chance of survival within 80 months (Fig. 4A). CHAC1 ($p = 0.00029$) (Fig. 4B), AHNAK2 (Fig. 4C) and PTP4A3 ($p = 0.0031$) (Fig. 4D) showed the same results; higher expression indicated a lower survival rate.

3.4. Nomogram predict the metastasis risk of UVM

To evaluate the potential values of the four co-expressed genes in UVM, we further analyzed GSE22138 dataset. The outcome was defined as metastases at 36th month after diagnosis. We first filtered HTR2B, CHAC1, AHNAK2, PTP4A3, tumor thickness, age, gender and tumor with retinal detachment (RD) as the possible risk factors for metastasis with LASSO regression (Fig. 5A and B), then age and gender were excluded. Forest plot has shown that tumor thickness and RD were significantly related to UVM metastasis in multivariate logistics regression model combined with selected variables (Fig. 5C). Then we established the nomogram for predicting the possibility of UVM metastasis in 3 years (Fig. 5D). ROC curve showed sufficient AUC (0.912) of our model (Fig. 5E), which suggests reliability of new model. Calibration curve is close to ideal curve, which has proved its high efficacy of this model (Fig. 5F). The model with four biomarkers (HTR2B, CHAC1, AHNAK2, PTP4A3) and other clinical factors could bring more clinical benefits than the model without biomarkers according to the decision curve (Fig. 5G).

4. Discussion

In our study, we have established a nomogram based on co-DEGs including HTR2B, CHAC1, AHNAK2, and PTP4A3 as well as clinical characteristics. Our study provides a predictive model and personalized risk estimation tool for assessment of UVM metastasis risk in 3 years.

Many efforts have been made to find the biomarkers for the metastases and prediction of prognosis. UVM has an alarming rate of metastases, which can be up to 31% in 5 years and 50% in 25 years [17,18]. However, the median survival rate is only about 12 months once the metastases were detected [19]. The curative effect is still limited though many new progresses in the field of metastatic UVM treatment have been made [20], which suggest we may shift emphasis to early prevention to prediction of metastasis [21]. Previous studies have explored prognostic biomarkers based on clinical and imaging manifestations, including age [22], gender [23], location of primary tumor [11], the size of primary tumor [24], vascular invasion [25]. In addition, molecule biomarkers for prediction are coming into prominence, due to the further understanding of molecule mechanism and biological behavior in UVM.

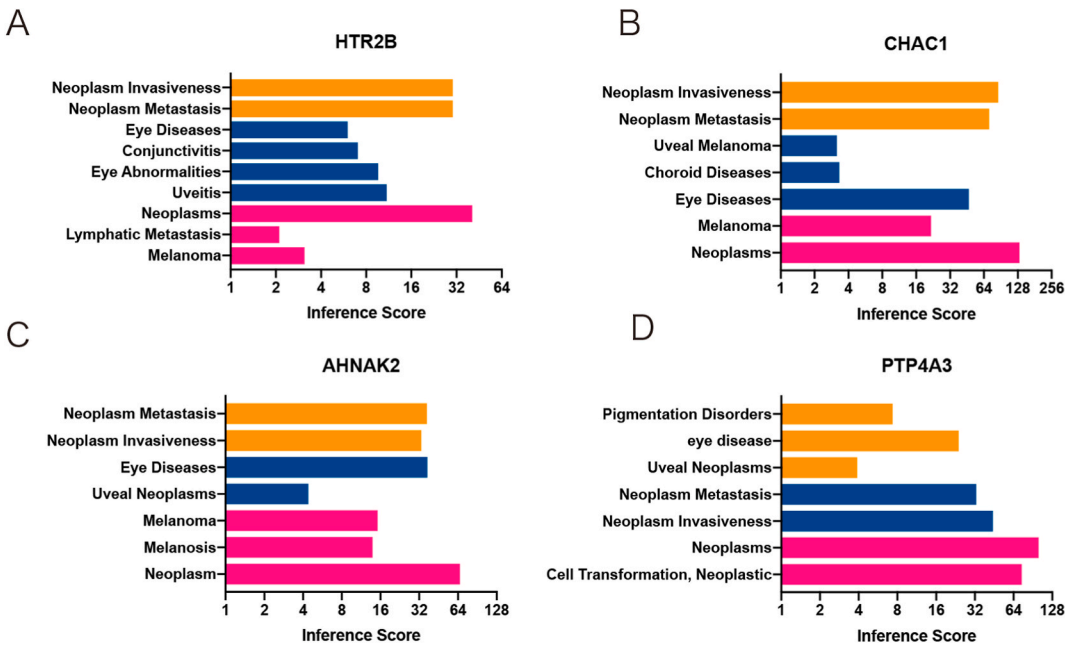

Fig. 3. A-D. CTD database showed these four genes were associated with cancer, eye diseases and metastasis.

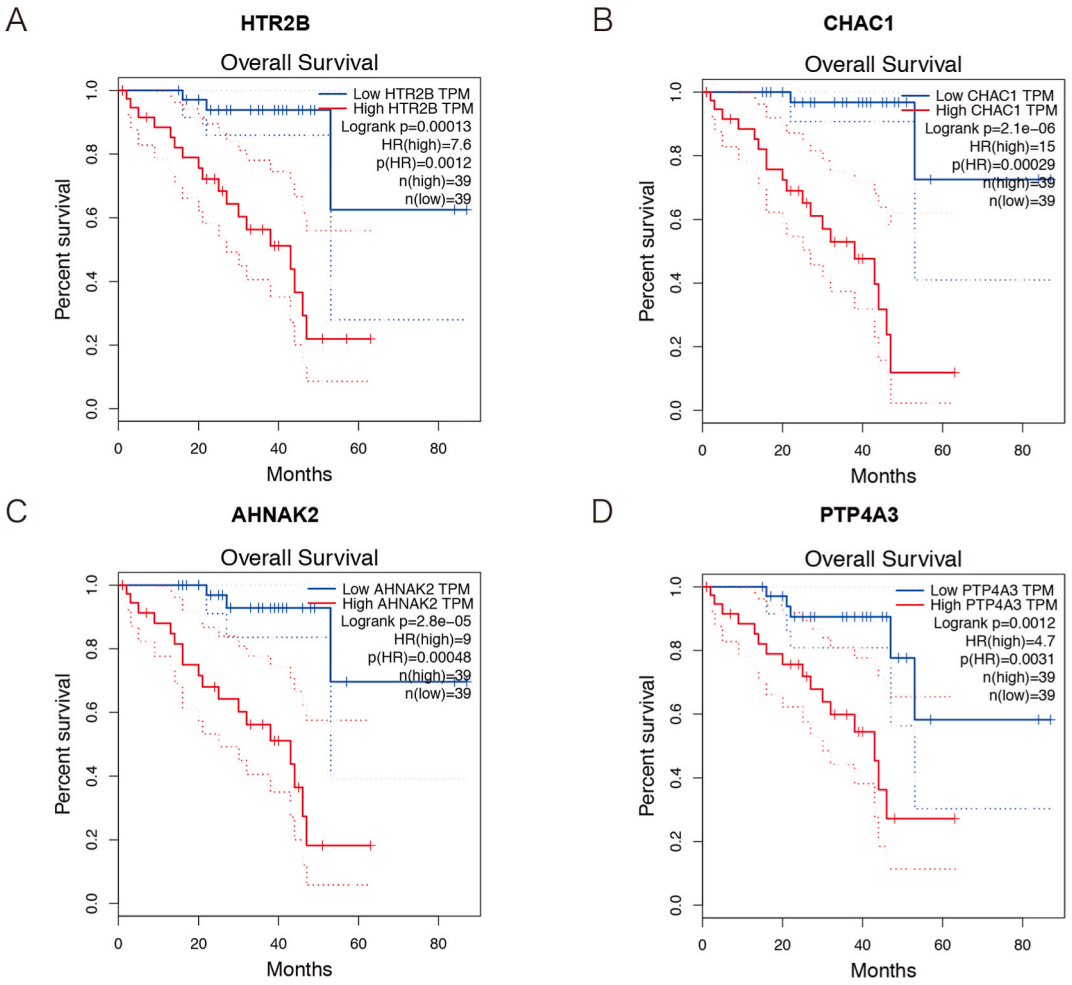

Fig. 4. A-D. The GEPIA database, an online tool based on TCGA database, revealed the expression pattern of HTR2B, CHAC1, AHNAK2, PTP4A3 in UVM.

Serotonin receptor HTR2B is known as a marker of tumor metastases, it can participate in cell proliferation and survival [26,27] by activating G proteins. Serotonin (also known as 5-HT), as the ligand of HTR2B, acts as a neurotransmitter and can be synthesized in the central nervous system, melanocytes, and melanoma cells [28,29]. Activated HTR2B can further activates G protein including guanine nucleotide-binding protein alpha Q (GNAQ) and GNA11 [30]. GNAQ was reported as the first mutation gene in UVM pathogenesis [31]. GNAQ and its paralogue GNA11 are involved in Gα11/Q pathway, which can activate ADP-ribosylation factor 6 (ARF6) and regulate the cell proliferation and growth [32]. GNAQ and GNA11 mutation decrease the guanosine triphosphate (GTPase) activity [32], and AKT/mTOR pathway [33], which leads to abnormal cell proliferation. Previous studies have reported HTR2B to up-regulated in UVM. HTR2B is the most reliable, predictive biomarker of liver metastases [27,34–36]. High levels of HTR2B are regulated by two transcription factors, namely NF1 and RUNX1. NFI can combine with several target sites of HTR2B promotors and exist as an activator of HTR2B. RUNX1 is also a repressing factor of HTR2B, and has three binding sites thereon [27]. In addition, post-translational modifications are involved in HTR2B expression, including the ubiquitin-proteasome system [34]; these lead to non-degradable HTR2B in metastatic uveal melanomas.

PTP4A3 may promote the aggressiveness of uveal melanoma cells by binding to ARF6. PTP4A3 has been proved to interact to ARF6 via co-IP, while ARF6 can activate multiple pathways to promote UVM cell proliferation and migration [37]. Besides, PTP4A3 may regulates integrin β 1 to promote UVM adhesion during migration [38].

AHNAK nucleoprotein 2 (AHNAK2) can also promote UVM migration by regulating HGF/MET pathway. Hepatocyte growth factor (HGF)/mesenchymal-epithelial transition factor (MET) signaling is a crucial pathway in UVM migration [39]. It promotes the epithelial-mesenchymal transition (EMT), which is an essential step of migration, invasion, and extravasation from primary tumor [39, 40].

AHNAK2 is reported to be associated with EMT as well [41]. Its expression is higher in high risk UVM, and its expression is positively correlated with HTR2B [42]. AHNAK2 can promote EMT and adhesion molecules (E-cadherin and β-catenin) through PI3K signaling pathway, and affect UVM cell proliferation and migration [43].

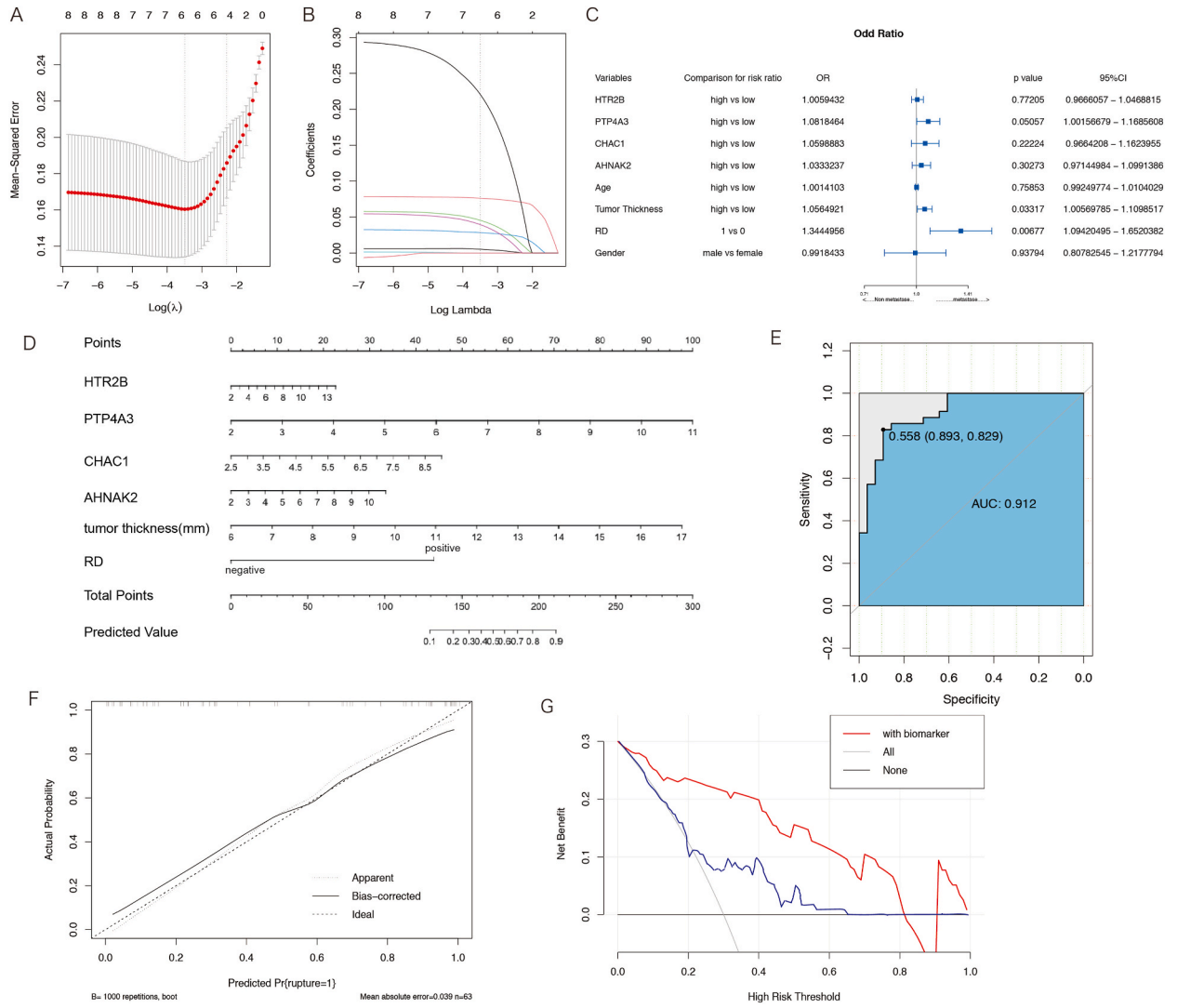

Fig. 5. A. The parameter lambda of Lasso model was selected by minimum criteria. The minimum λ was 0.02522957. B. Lasso regression showed 6 variables selected by the λ value (the minimum λ was drawn as the vertical line). C. Forest plot of the selected variables illustrated the prediction of tumor metastasis. The results in forest plot was calculated by logistic regression. D. A nomogram of five selected variables was built for predicting the risk of tumor metastasis. E. ROC curve of the nomogram model in a validation set, AUC = 0.912. F. Calibration curve of the model. G. Decision curve of the model. Red curve represents the net benefits of the model with biomarkers (HTR2B, CHAC1, AHNAK2 and PTP4A3) and clinical characteristics (tumor thickness and RD). Blue curve means the net benefits of the model without biomarkers (HTR2B, CHAC1, AHNAK2 and PTP4A3), with only clinicopathological factors.

Cation transport regulator-like protein 1 (CHAC1) is another biomarker of poor prognosis of UVM(21). It is a ferroptosis-related gene [44], and promote cell survival and proliferation by regulating the apoptosis [45]. CHAC1 is upregulated in UVM tissue, knockdown of CHAC1 can decreased the growth and migration of UVM cells [46].

Compared to existing UVM prognosis studies, our study showed some advantages. First, we combined both molecule biomarkers and clinical manifestations, to provide a prediction tool for UVM metastasis rate. Nomogram has been widely used in tumor for prediction of prognosis, which can provide a quantitative probability of a clinical outcome by integrating different variables [47,48]. The advantage of nomogram is that is a graphic tool and can be calculated quickly without a computer or a calculator. Though the advanced technology has brought more complicated and accurate models, nomogram still has its own advantages. It is easier and more visualized for clinician to understand each predictor's impact on the outcome [49]. Several studies have compared nomogram to other models. A study on predicting the survival of non-small cell lung cancer patients found nomogram provided more reliable prognostic assessment than machine learning

with a higher accuracy of 0.85 [50]. In tongue cancer, machine learning can provide more reliable prognostic information, while nomogram is more direct in estimating the outcome and helping patients and clinicians for making decisions together [51]. Therefore,

nomogram is not inferior to other predictive models. However, machine learning and AI requires large sample size for training, considering to the small sample size, nomogram will be a better way. Nomogram is also used in uveal melanoma. Zeng et al. has developed a nomogram for survival prediction based on several clinical characteristics [52]. However, our model showed higher AUC (0.912) than Zeng et al.'s study (AUC = 0.771) in ROC curve, which means our advantages in accuracy.

In addition, the model with four biomarkers (HTR2B, CHAC1, AHNAK2, PTP4A3) and other clinical factors could bring more clinical benefits than the model without biomarkers according to the decision curve. Most of the prediction model of uveal melanoma was based on clinical features, such as age, gender, tumor size, and involvement of adjacent tissue [52–54]. Biomarkers are also important indications for tumor detection and prognosis, such as CA125 for ovarian cancer [55], AFP for hepatocellular carcinoma [56]. We are still lack of sensitive biomarkers in uveal melanoma to monitor the progress of disease, which leads to its poor prognosis [57]. Our model would provide new perspectives in the diagnosis and prognosis of uveal melanoma by combing biomarkers and clinical features.

There are still few limitations in our study. We did not conclude other risk factors such as region, skin color, radiation exposure in our study [58], since the datasets lacked this information. In addition, our model is lack of external validation due to the uncomplete clinical data. Though we have done internal validation and the results showed the model was relatively reliable, it will be better if the model is tested by another dataset. Besides, our data was assessed from public database, and we are lack of original data. The data only presents the metastases situation at 36th month (3 year) of their visit. We will collect our own data in the future and expect our future study will make up for these shortages.

5. Conclusion

We established a nomogram based on co-DEGs of two UVM sequencing datasets, including HTR2B, CHAC1, AHNAK2, and PTP4A3. These biomarkers are combined with clinical characteristics, which provides a comprehensive predictive model and personalized risk estimation tool for assessment of 3-year metastasis risk of UVM.

Ethics statement

Ethical approval and consent were not required as this study was based on publicly available data. The need for informed consent was waived by the ethical committee of Shanghai General Hospital.

Study approval statement

Ethical approval and consent were not required as this study was based on publicly available data. The need for informed consent was waived by the ethical committee of Shanghai General Hospital.

Consent to participate statement

Patient consent were not required as this study was based on publicly available data.

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Author contribution statement

Xiaohuan Zhao; Xinyue Zhu: Conceived and designed the experiments.

Wenjia Liu; Xiaodong Sun: Conceived and designed the experiments; Analyzed and interpreted the data.

Feng Lin: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Yimin Wang; Minyue Xie: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jieqiong Chen: Analyzed and interpreted the data.

Xiaonan Sheng: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Yuwei Wang; Bing Lu: Contributed reagents, materials, analysis tools or data.

Ting Zhan; Xiaoling Wan: Wrote the paper.

Data availability statement

The authors do not have permission to share data.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18956.

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