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# Integrated Analysis of a Gene Signature for Prognosis Prediction in Uveal Melanoma

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**Abstract**

**Background:** Uveal melanoma (UVM) is a rare disease but most common primary intraocular malignant tumor among adults. The destructive degree of this tumor is high with poor prognosis. Therefore, it is urgent to discover novel prognosis biomarkers of uveal melanoma.

**Methods:** mRNA expression patterns were examined in 80 UVM samples collected from the Cancer Genome Atlas. Univariate Cox regression and Lasso regression analysis were used to evaluate the associations between genes and survival time. A risk score based on genes was calculated to assess the predictive value. ROC (Receiver Operating Characteristics) analysis was applied to assess the sensitivity of survival prediction. Pseudogene–miRNA–mRNA network was visualized by Cytoscape.

**Results:** With univariate Cox regression and Lasso regression analyses, the expression level of six genes (*CLNS1AP1*, *MAST4-AS1*, *MIR6870*, *NIPA2P1*, *PADI3*, and *SPRR4*) was significantly correlated with survival time of UVM patients. Based on risk score, patients were divided into a high-risk and low-risk group. Interestingly, the identified genes were nearly just associated with the survival risk in UVM. Moreover, we predicted miRNAs (hsa-miR-580-3p, hsa-miR-520a-5p, and hsa-miR-525-5p) that bound to the pseudogene *CLNS1AP1*. *BCL2L1*, *FOXO3*, *FOXL2*, *TWIST1*, *PARP1* and *VDR* were identified as the potential targets of *CLNS1AP1*.

**Conclusions:** *CLNS1AP1*, *MAST4-AS1*, *MIR6870*, *NIPA2P1*, *PADI3*, and *SPRR4* were identified as potential biomarkers of UVM. We constructed the pseudogene–miRNA–mRNA network of *CLNS1AP1* and discussed its potential mechanism. This work may have important implications in the understanding of the potential prognostic value of gene-based signatures in UVM.

**Keywords:** uveal melanoma; risk score; *CLNS1AP1*; pseudogene–miRNA–mRNA

## 1. Introduction

Uveal melanoma (UVM) is the most prevalent primary intraocular tumour in adults with 6-7 in 1 million population (1, 2). UVM is an uncommon condition in humans. If the cancers are detected and managed locally until metastasis, the 5-year survival rate is over 90 percent. Unfortunately, nearly 50 percent of patients have systemic metastasis, with the most frequent metastases in the liver, accompanied by the lungs and skeleton (3, 4), which indicates that these patients have a median survival period of 2-15 months (5). The tumour malignancy is elevated and the rear of the eye is the primary site. It can be moved to the liver quickly (85%) through blood flow and the prediction is horrible in this situation.

Earlier research identified some genes with UVM prognostic benefit. BAP1 (BRCA1-associated protein 1) mutations have been shown to be related to metastatic mucus, greater tumour scale, and participation of ciliary body (6-8), in uveal melanoma cells. A possible goal for immunotherapy in cutaneous melanoma is PRAME (preferably presented as an antigen of melanoma). It is also evidently correlated favourably with tumour diameter, metastasis and survival rate in uveal melanoma cells (9, 10). Mutations of GNAQ (G Protein Subunit Alpha Q) and GNA11 (a GNAQ paralogue) are known and are stated to contribute significantly to UVM 's production in approximately 83 percent of uveal melanomas. It had no connexion, however, with the metastasis survival

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forecast (11, 12). The weighted gene co-expression network analysis (WGCNA) was conducted to explore the interaction between molecules and clinical characteristics. The data indicated that there could be a significant function for the recurrence of UVM in the established hub genes (slic17A7, ntrkrk2, Abtb1, and AdpRHL1). While several UVM genes have been identified, they remain subject to verification of their specificities and predictive ability.

In this research, we examined gene sequence data for uveal malignant melanoma using the publicly released results from the TCGA project. We have developed a prognostic risk score based on a genetic signature through Cox regression analyses and Lasso regression, to display the connexion between genes and UVM prognosis. We observed that six genes described were linked to a particular risk of UVM survival. In addition, we first explored and addressed the possible function of the pseudogen-miRNA – mRNA interaction phase CLNS1AP1, in UVM.

## 2. Methods

### 2.1. Collection of publicly available data from TCGA

The TCGA database open to the general public has been downloaded to counts and FPKM (fragments per kilo-base of exon per million reads mapped) for gene sequencing of uveal malignant melanoma. Using the Cuffdiff 2.2.1 to convert FPKM data into TPM data (transcripts per million reads), and ENSG has been converted into the gene symbol after removal of low expression gene data (max CPM < 1. Survival statistics have been accumulated and 80 samples have been collected. The 80 samples were then split into a training set (N = 40 for crucial gene identification) and a test set (N = 40 for gene signature verification).

### 2.2. Identification and selection of prognosis-related genes

The association between a significant number of the expressed genes and the whole patient lifespan was examined in the training collection. The Univariate Cox regressions were used to test gene-survival relationships by R module survival (v2.44-1.1 suvival). In Cox proportional threat models, HR and 95% CI were calculated. Statistically relevant p-value less than 0.001 was found (14).

We have further screened forecast-related genes and identified six candidate genes via the Lasso regression study with v2.0-18 for enhancement of the feability and reliability of gene-based clinical prognosis.

### 2.3. Risk score formula establishment and validation

A risk score model was built in Cox's regression analysis of the training sample, which included these genes linked to prognosis and weighted their prediction of regression. This method has been used to measure each gene's risk score in the training sequence and the resulting median risk score is used as a cut-off value. The genes have been split into a high-risk and low-risk category. The characteristic curve of gene handling was obtained by combining R with the ROC data package (suvival 2.44-1.1). The survival differential of the low-risk community to the high-risk group was calculated using the Kaplan-Meier calculation and a logarithmic rating test to measure the discrepancy was used.

### 2.4. Kaplan–Meier survival analysis

For genes, Kaplan–Meier overall survival analysis and log-rank test were used to evaluate the statistical significance of survival differences between the low-risk and high-risk groups using starBase v3.0.

### 2.5. Construction of pseudogene–miRNA–mRNA network

The target microRNAs of pseudogene *CLNS1AP1* were identified using starBase v3.0. Target genes for identified microRNAs were retrieved with the miRTarBase tool. Pseudogene–miRNA–mRNA network was visualized using Cytoscape v3.4.0.

### 2.6. Gene ontology & KEGG pathway enrichment analysis

The identification of hub genes for target genes and enrichment analysis for target genes were performed using the ClueGO v2.3.5 plugin of Cytoscape v3.4.0. The p-values of GO items/pathways less than 0.05 were considered as significant.

## 3. Results

According to the study procedure (Fig. 1), the expression profiles of 13787 genes were collected for 80 patients' samples (Uveal Melanoma) from the TCGA database.

### 3.1. Identification of prognosis-related genes

We downloaded the counts and FPKM data of UVM and removed all the gene data with Low speech dependent on our criterion for filtering (Max CPM < 1). The data for review was ready after translating the data from FPKM (Fragments Per Kilobase Million) to TPM (Transcripts Per Million). In

a testing package and evaluation range, a total of 80 samples were further randomised. In the training sample, nine substantially different expressed genes were defined (Fig. S1, p<0.001, Table S2). The Cox regression study is univariable. Regression analysis was conducted based on these nine genes for further screening and six genes were eventually established as possible genes linked to UVM risk of survival (CLNS1AP1, MAST4-AD1, MIR6870, NIPA2P1, PADI3, and SPRR4). S2).

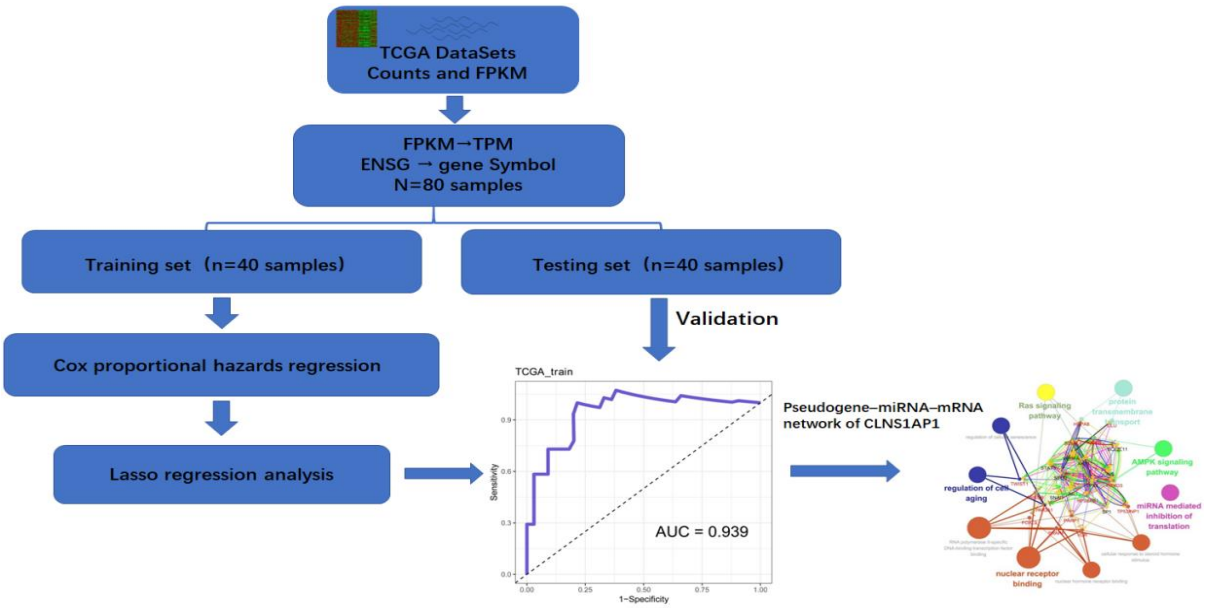


Figure 1. **Flow-chart of the study. Abbreviations: TCGA, the Cancer Genome Atlas; FPKM, Fragments Per Kilobase Million; TPM, Transcripts Per Million; AUC, the Area Under Curve.**

*Table 1.* **Prognosis-related genes screened by lasso regression analysis in the training set (N= 40).**

| Final genes | Active Coefficients |
|---|---|
| CLNS1AP1 | 0.013933464 |
| MAST4-AS1 | -0.01479341 |
| MIR6870 | 0.019720808 |
| NIPA2P1 | 0.054062975 |
| PADI3 | 0.020097776 |
| SPRR4 | 0.005367937 |

### 3.2. Risk score based on the signature of the six genes and prognosis of UVM

We used a risk assessment method dependent on their cox coefficients to extensively analyse the association between these six genes and the prognosis of UVM. The risk scores for each patient is determined for each training range (Cox coefficients * expression value of and gene). Data indicates that patients can be categorised as high-risk groups (N = 20) and low-risk groups (N = 20) depending on the risk ratings. UVM patients have a poor association with risk scores during recovery periods (Fig. 2). Many tragic events were high-risk, whereas patients with extended recovery were susceptible to a lower risk classification. Higher levels of expression of these genes in the high-risk population are typically found (Fig. 2). Compared to people in high-risk groups in the Kaplan-Meier curve (Log-rank test p<0.0001) the patients in the low-risk community have a longer survival period (Fig. 2). ROC analyses were conducted for the six-gene risk score in order to test the sensitivity and accuracy of the survival forecast. As in the photo. Three, the AUC (curve area) is 0.939. The risk score dependent on the signature of the selected 6 genes indicated that the prediction of UVM will possess an outstanding predictive ability.

### Validation of the six-gene signature-based risk score for survival prediction in UVM

In order to confirm the results of this risk score, the sum set and the test set were validated. The patients in the entire set were divided into high risk (N = 40) and low risk (N = 40). They were divided by optimal cut-off point (-0.002) according to the same formula (Figure 4B). Also, patients were classified in the test set-in high-risk groups (N = 23) and low-risk groupings (N = 17), with the optimum cut-off item (0) (Fig. 4A). The Kaplan-meier overview and test set curves shows that the recovery period for low-risks patients is considerably higher, compared with the

high-risk community (p < 0,0001), in accordance with training set data. A high-risk grouping of 31 patients and a low-risk category of 121 patients resulted in an ideal cross-sectional research set classification. In addition, both the comparison AUC (0.756) and total (0.826) values have also been determined (Fig. 3). The survival study provided the same findings as patients with low risk had slightly increased survival period as patients with high risk, considering the sample size unbalancing of each category (p<0,0001) (Fig. 4B).

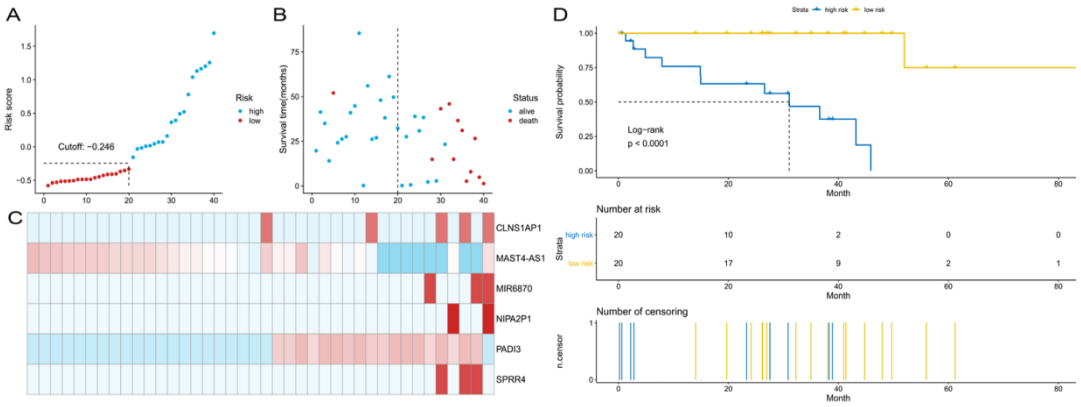


Figure 2. **Training Set Six-Ge Risk Score Review (N=40). (a) Distribution of the six gene signature risk score in an exercise package. (b) Duration of life time for patients. c) the gene expression profiles of the nominee heat index. (d) The patients on the training range were classified by Kaplan-Meier classification into low-risk and high-risk categories.**

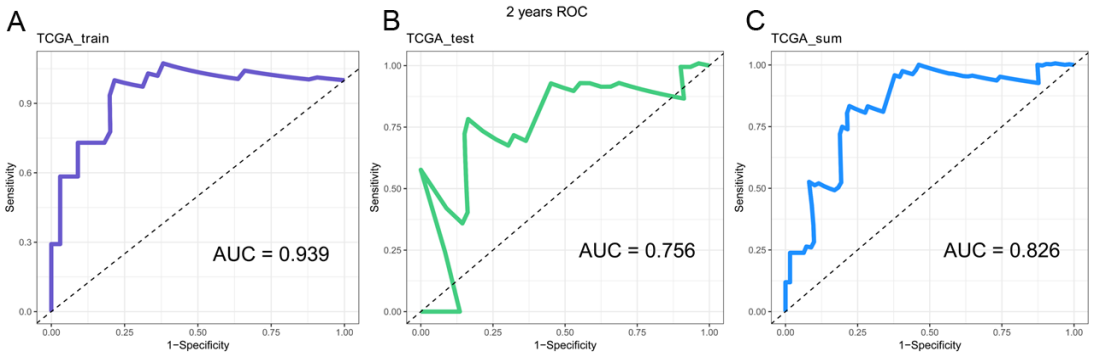


Figure 3. **Two-years ROC analysis of the sensitivity and specificity of the survival time with the six-gene signature-based risk score in the training (A), testing (B) or sum (C) group.**

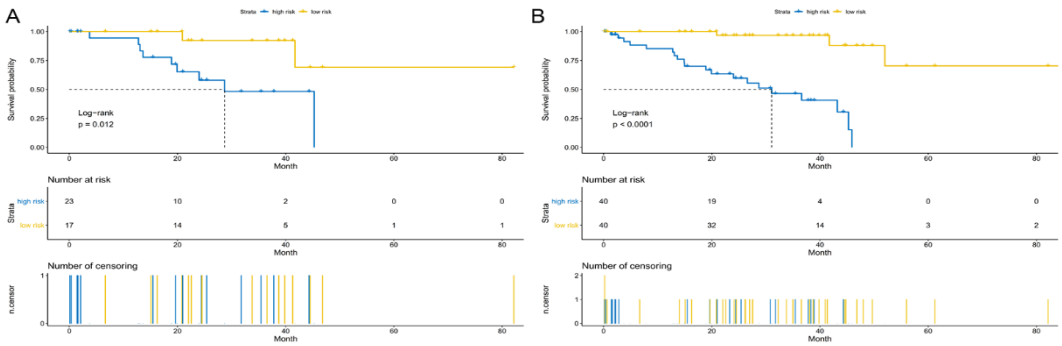


Figure 4. **Six-gene risk score analysis in the testing (N = 40) and sum set (N = 80). (A) The patients of the testing set were divided into low-risk and high-risk groups following Kaplan-Meier analysis. (B) The patients of the sum set were also divided into low-risk and high-risk groups according to Kaplan-Meier analysis.**

### 3.3. The specificity of the six-gene signature in UVM

We used the starBase v 3.0 analyzer online to examine the factor of our study (15, 16). Because the collected data were identical to the starBase v3.0, our consequences would be comparable to those of starBase v3.0. In order to discuss overall Survival for the six target genes, a "Pan cancer survival analysis" was adopted. The figure shows that each of the target genes could be divided into a high-risk group and a low-risk group. There was, obviously, a statistically significant difference (Log Ranking $p < 0.01$) between the two groups.

In addition, the survival analysis of target genes across 32 types of cancers was further investigated based on the starbase v3.0. It is interesting to note that our target genes are almost exclusively linked to the risk of UVM survival (Table S3). For instance, the CLNS1AP1 expression pattern did not relate to the prognostics of 29 other types of cancer, except for uvial melanoma (p=0.0035), kidney cell clear cell carcinoma (p=0.011) and adenocarcinoma stomach (p=0.049). The six candidate genes can predict UVM survival risk specifically. It suggests.

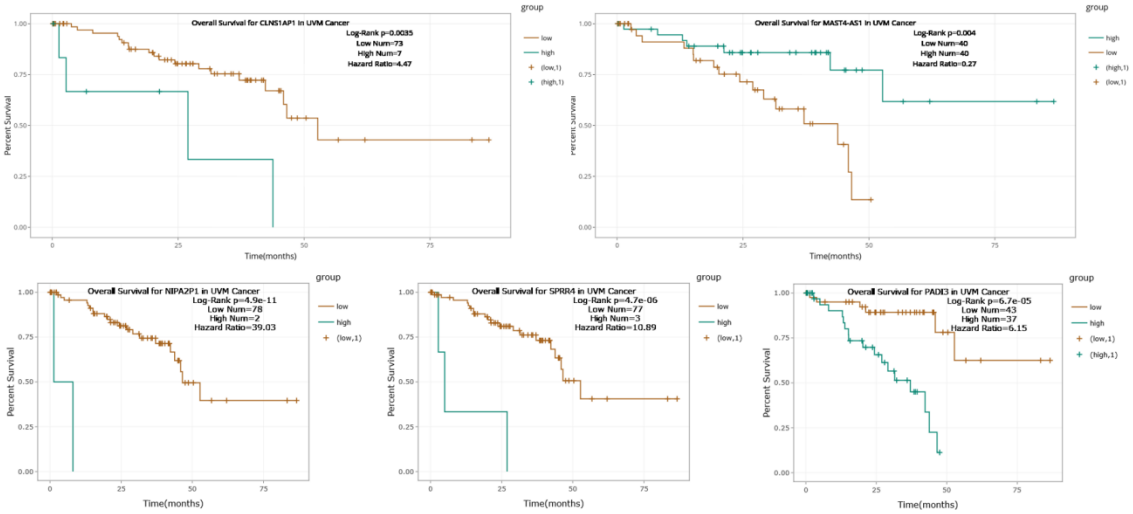


Figure 5. **The gene survival analysis for *CLNS1AP1*, *MAST4-AS1*, *NIPA2P1*, *SPRR4*, and *PADI3* in UVM using starBase v3.0.**

### 3.4. Pseudogene–miRNA–mRNA regulatory network of CLNS1AP1

As far as we are aware, there have been no pseudogenic studies in the CLNS1AP1 gene function. CeRNA is an important pseudogen regulatory mechanism in which pseudogenes act as sponges for RNA in mirRNAs and regulate the expression of target genes by competing with common miRNA binding. StarBase v3.0 has been used to classify possibly pseudogenic miRNAs. The candidate miRNA was identified for CLNS1AP1, hsa-miR-580-3p, hsa-miR-520a-5p, and hsa-miR-525-5p (Table 2). The miRTarBase then has been used to predict these candidate miRNAs' target genes. A total of 418 genes have been identified (Table S4) and Cytoscape has then displayed the pseudogen-miRNA-mRNA regulatory network. Thus, through regulating those target genes, CLNS1AP1 may exercise biological functions.

*Table 2.* **miRNAs targeting *CLNS1AP1* as predicted by starBase v3.0.**

| miRNA | GeneID | Alignment |
|---|---|---|
| hsa-miR-520a-5p | ENSG00000213335 | **Target: 5' cuugguUAGAUAGCUCUGGAu 3'**<br>**\|\| \| \|\|\|\|\|\|\|**<br>**miRNA : 3' ucuuucAUGAAGGGAGACCUc 5'** |
| hsa-miR-525-5p | ENSG00000213335 | **Target: 5' cuugguuagAUAGCUCUGGAu 3'**<br>**\|\| \|\|\|\|\|\|\|**<br>**miRNA : 3' ucuuucacgUAGGGAGACCUc 5'** |
| hsa-miR-580-3p | ENSG00000213335 | **Target: 5' agcucUGGAUUAGGAUUCUCAc 3'**<br>**\|\|: \|:\| \|\|\|\|\|\|\|**<br>**miRNA : 3' ggauuACUAAGUAGUAAGAGUu 5'** |

The enrichment analysis of the GO and KEGG pathways with ClueGo was done to investigate the potential functions of CLNS1AP1 in the process of UVM ($p < 0.05$). 10 GO terms have been added for CLNS1AP1 (Fig. 6A, Table S5). Ras signalling pathway (KEGG 27.02.2019; p = 2.99E-4) and AMPK (AMP protein kinase) signalling pathway have been among the largest enriched gene sets (KEGG 27.02.2019; p = 2.24E-4). The relationship between ClueGo-identified hub genes (Table S6) and a UVM risk of survival was also examined. In the 24 hub genes we found that the UVM prognosis correlates significantly between the BCL2L1, FOXO3, Foxl2, TWIST1, PARP1 and VDR genes alone (see Fig. 7). These findings show that CLNS1AP1, through pseudogene-miRNA-mRNA mechanism, can control the expression of its downstream genes (Fig. 6B).

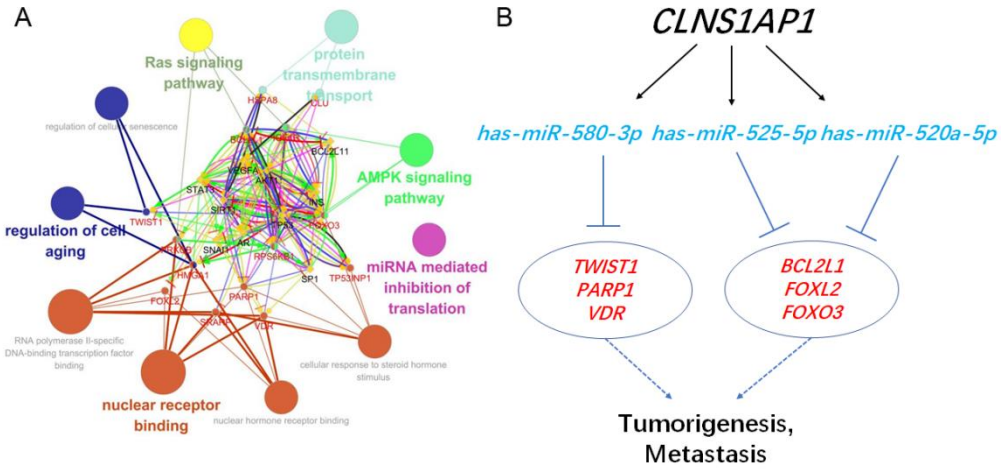


Figure 6. **CLNS1AP1 's probable function in UVM. (a) Analysis of functional enrichment of hub genes identified as target genes of miRNA. ClueGo v2.3.5 Cytoscape v3. 4.0 plugin was used for GO- and KEGG pathway enhancement analyses for CLNS1AP1 genes. In short, there were enriched 10 terms / pathway ($p < 0.05$). For the statistical test and correction method respectively, enrichment / depletion (twidely-sided hypergeometric tests) and Bonferroni Step were used. The pathways were substantially enriched in Ras (p=2.99E-4) and in AMPK (p=2.24E-4). (b) Scheme of the possible UVM ceRNA regulatory network mediated by the CLNS1AP1.**

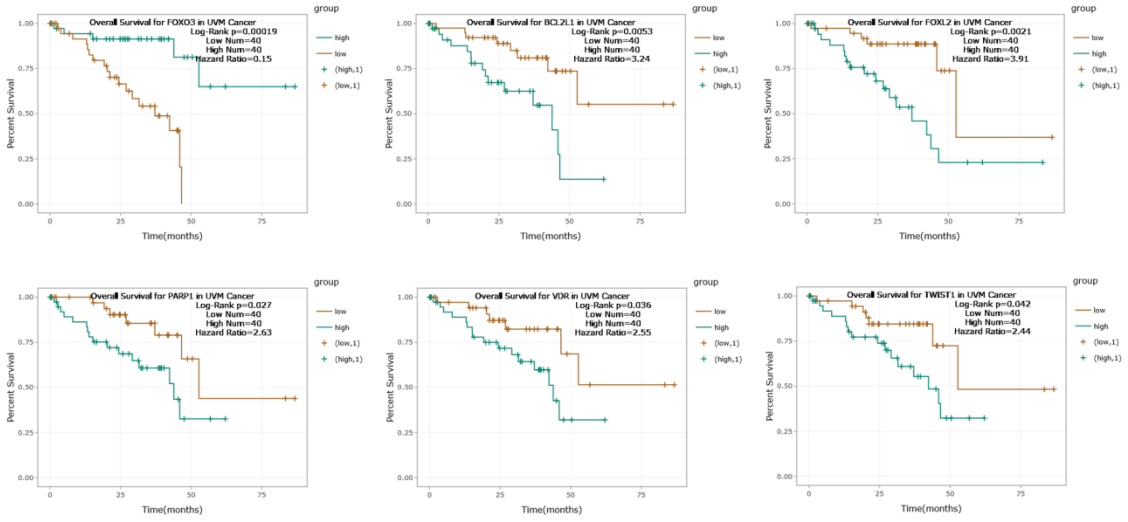


Figure 7. **Survival analysis of the hub genes for *FOXO3*, *BCL2L1*, *FOXL2*, *PARP1, VDR* and *TWIST1* in UVM using starBase v3.0.**

## 4. Discussion

Uveal melanoma is an extremely deadly disease, and it is particularly necessary to investigate genes linked to the likelihood of UVM survival. In this scenario, we have identified six genes that are correlated with UVM patients in training sessions

(CLNS1AP1, MAST4-AS1, M IR6870, NIPA2P1, PADI3, and SPRR4). We established a six-genes-based risk ranking according to their coefficients arising from the univariate Cox regression. Furthermore, ROC analysis verified its prognostic importance. In both the internal test collection and the full sample, both the risk score model and the optimum cut-off point were further checked. Of interest, the six genes were correlated significantly with the UVM survival period. The six genes have been proposed for UVM prognosis as a biomarker. We know the possible function CLNS1AP1 and NIPA2P1 play in cancer prognosis is established by first research. As far as we know. The findings show that these candidate genes may play a key role in the production and prognosis of UVM.

CLNS1AP1 and NIPA2P1 are pseudogenes of the six nominee genes.

MAST4-AS1 belongs to ncRNAs, microRNAs are MIR6870, and functional genes are PADI3 and SPRR4. Amazingly, functional studies of all these genes are lacking. Some studies have shown the involvement of PADI3 in the formation of a hair-shaft (17). Uncombustible hair syndrome may result in mutations in the corresponding protein (18). A myEIRA study and meta-analysis (Malaisian Epidemiological Investigation of Rheumatoid Arthritis) showed that the declines of peptidylarginine in various Asian populations are associated with rheumatoid arthritis (19). The TSPRR4, a member of the SPRR (small proline rich proteins), showed low expression skin or other squamous stratified epithelia but was inducted from UV light in physiological conditions; the SPRR4 is included in adaptive response to stresses in the environment (20, 21). The amplification of MIR6870 at genome level was observed in the Wilms tumour (22). The enhanced MIR6870 is suggested to be a major event in Wilms tumour development. There have been no functional studies yet for CLNS1AP1 and NIPA2P1.

MicroRNAs silence genes by binding mRNAs, whilst ceRNAs (competitors in endogenous RNAs) can bind to micro RNAs by using MREs to regulate the microRNA-caused gene silencing (23, 24). Pseudogenes have long been regarded as "junk DNA" and have no known function (25). In recent years, many Pseudogenes have evidently served as a new class of long non-coding RNAs working with a ceRNAs mechanism in disease (26). OCT4-pg4, an OCT4 pseudogen, in HCC (hepatocellular carcinoma) is abnormally activated. MIR-145 can targeted OCT4-pg4 and OCT4 directly. However, OCT4-pg4 acted to protect the spongiform OCT4 from miR-145 inhibition in hepatocellular carcinoma as a natural microRNA sponge (27). The transcript level of PTEN was found to increase with PTENP1 pseudo genes (phosphate genes and tension homologies deleted on Chromosome 10) due to the competitive binding of miR-17, miR-19, miR-21, miR-26, and miR-214 in prostate cancer (28). (28).

No accessible CLNS1AP1 and NIPA2P1 analysis was performed in our sample. We have developed a pseudogene-miRNA / mRNA-interaction network to predict CLNS1AP1 's functional functions. The target microRNAs were described as hsa-miR-525-3p, hsa-miR-520a-5p, and 418 genes may serve as the CLNS1AP1 possible regulatory goals. The 418 targets were identified by a practical improved study of GO items / pathways aligned with the CLNS1AP1. The Ras signalling pathway and AMPK signalling pathways improved much of the genes. One of the most widely triggered signal transdution routes in cancer are the Ras and AMPK signalling channels. Ras provides hormonal signals to cells in a physiological circumstance and controls cell development. If the Ras signals remain triggered for a long period, tumours can grow and mature (29). AMPK is a central mediator for cellular homeostasis energy and has emerged as a potential metabolic tumour suppressor and cancer therapy goal (30, 31). We have also established 24 CLNS1AP1-microRNA-regulated hub genes. Almost all of the hub genes known have been recorded as the key genes involved in the cancer process. However, only six genes have demonstrated important correlations with the prognosis of UVM (BCL2L1, FOXO3, FOXL2, TWIST1, PARP1, and VDR). Twist-related protein 1 (TWIST1), for example, is a transcription factors for epithelial-mesenchymal processes. miR-186 prevents proliferation of the tumour by TWIST1 inhibition in glioblastoma and cholangiocarcinoma cell (32). The TWIST1 gene was substantially related to the level of development and the scale of tumours in the oesophagus squamous cell carcinoma (33). In UVM, the high expression of TWIST1 was stated to be correlated with a bad pronostic of a different tumour cohort (34). It was proposed that the irregular signals TWIST1 was a critical point for tumours. We also find that TWIST1 is correlated with the UVM survval chance by way of survival association tests. In addition, TWIST1 has been tested by a reporter assays and Western blot as the downstream gene of hsa-miR-580-3p (Table S4). Finally, CLNS1AP1 can change CL2L1, FOXO3, FOXL2, TWIST1, PARP1 and VDR expression patterns by acting as a "sponge" microRNA target set (HSA-miR-580-3p, HsA-miR-520a-5p and HSA -MIR-). *525-5p*) in UVM.

This research finds many genes to be important for determining uveal melanoma pronostics. Nevertheless, all six genes that were known were previously shown to have no role in uveal melanoma pathogenesis. Usually 2 of them were not included in the prediction of any form of cancer (CLNS1AP1 and NIPA2P1). The remaining four genes are ncRNA, microRnA or functional genes and none of these have been historically attributed to uveal melanoma and have carried out functional studies. In comparison, the lack of a UVM dataset restricts validity of an individual sample. The research therefore offers only some genes that are connected to uveal melanoma 's prognosis and growth. More studies are needed to explore the function and pathways of these genes in uveal melanoma pathogenesis.

**Competing interests**

We declare that we have no financial and personal conflicts with other people or organizations.

**Fund**

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**Availability of data and materials**

All data and materials can be provided upon request.

**Ethics approval and consent to participate**

Not applicable.

**Reference**

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