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CIB1 as a Potential Diagnosis and Prognosis Biomarker in Uveal Melanoma

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Abstract

Background: Uveal melanoma (UVM) is the most common primary intraocular tumor in adults. However, identification of the effective biomarker for the diagnosis and treatment of UVM remains to be explored. Calcium and integrin-binding protein 1 (CIB1) is emerging as an important factor in tumor progression. **Purpose:** To determine the contribution of CIB1 in the diagnosis of UVM. **Method:** Immunohistochemical staining is used to detect the CIB1 expression level, while Gene Expression Profiling Interactive Analysis 2 (GEPIA2) and UALCAN online tools were used to analyze patient survival and CIB1 correlation genes in UVM. Integrative analysis using STRING and GeneMANIA predicted the correlated genes with CIB1 in UVM. **Results:** CIB1 expression level in UVM was significantly enhanced when compared with that in paracancerous tissues. A higher CIB1 expression level resulted in a significantly worse disease-free survival as well as overall survival. Moreover, the survival probability of patients was associated with body weight and gender of the patients with UVM. The correlated genes with CIB1 in UVM, and the similarity of the genes in UVM expression and survival heatmap were verified. Furthermore, Gene ontology enrichment analysis revealed that CIB1 and its correlated genes are significantly enriched in ITGA2B-ITGB3-CIB1 complex, regulation of intracellular protein transport and regulation of ion transport. **Conclusions:** Our novel findings suggested that CIB1 might be a potential diagnostic predictor for UVM, and might contribute to the potential strategy for UVM treatment by targeting CIB1.

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Keywords

CIB1, Uveal Melanoma, TCGA, Prognostic Biomarker, Patient Survival

1. Introduction

Melanomas are originating from melanocyte transformation and represent a fatal form of highly malignant cancer. There are two subtypes of melanomas have been classified, cutaneous melanoma (SKCM) and ocular melanoma (OM) [1]. As the most frequent (85%) primary intraocular malignancy arising in adults, uveal melanoma (UVM) is a clinically distinct and specifically lethal subset of melanoma deriving from eye melanocytes [2]. The large majority of UVM arises from the uvea (95%), comprising the posterior uvea (choroid 90% and ciliary body 5%) and the anterior uvea (5%) of eye [3]. Little is understood concerning the molecular pathogenesis of UVM in contrast to SKCM, particularly in associated genes or tumor suppressor pathways owning to perplexing host and environmental factors influencing the aggression of UVM. Currently, the treatment of localized UVM involves radiation, immunotherapy, laser therapy, or surgery excision [4] [5] [6] [7]. However, there are no effective therapeutic strategies for patients with UVM [1], and the prognosis of metastatic UVM remains not adequate, with an overall survival of less than one year in most objects [8]. Cytogenetic has identified several alterations, which include monosomy 3, chromosome 6 abnormalities and *MYC* amplification at 8q24 are strong diagnostic and prognostic markers in UVM [9] [10], to investigate the novel prognosticators of UVM and an enhanced interpretation of the potential molecular mechanisms and gene networks associated in the development and prognosis of UVM are still required.

Calcium and integrin-binding protein 1 (CIB1) is an EF-hand calcium-binding protein, which is ubiquitously expressed and is associated with various human cancers [11]. Accumulating evidence has fuelled the conclusion that CIB1 performs crucial roles in facilitating cell survival, proliferation and migration, thereby mediating tumor growth and tumor-induced angiogenesis [12] [13] [14]. In particular, Chung *et al.* recently documented that CIB1 depletion with docetaxel or TRAIL enhances triple-negative breast cancer cell death [15]. However, the potential diagnostic value of CIB1 on UVM is unclear.

In the present study, CIB1 expression level of the typical patients with malignant UVM was analyzed. Moreover, bioinformatics analysis from The Cancer Genome Atlas (TCGA) by UALCAN interactive web-portal [16] and Gene Expression Profiling Interactive Analysis 2 (GEPIA2) online tools [17] were used to identify the critical role of CIB1 gene in UVM diagnosis and prognosis. CIB1 expression level in UVM, effect of CIB1 expression level on patient survival, promoter methylation levels of CIB1 in UVM and the correlated genes with CIB1 in patients with UVM were also presented.

The STRING database (http://string-db.org) provides a critical evaluation and integration of protein-protein interactions (PPI), including physical and functional relevance [18]. GeneMANIA (http://genemania.org) is a flexible user-friendly website for generating hypotheses regarding gene function, analyzing gene lists and prioritizing genes for functional assay [19]. Metascape tool (http://metascape.org) provides a resource for biologists for the analysis of systems-level datasets [20]. Thus, CIB1 PPI network and Gene ontology (GO) enrichment analysis were highlighted by using STRING/GeneMANIA and Metascape, respectively. Taken together, the diagnostic and prognostic role of CIB1 in UVM was identified. Thus, the results from the current study might advance the development of antagonist CIB1 strategies for patients with UVM.

2. Materials and Methods

2.1. Patients and Ethics Statement

Patients were recruited from the Ophtalmology department of the Second School of Clinical Medicine & Jingzhou Central Hospital (Jingzhou, China). Inclusion criteria: 1) Deformities, diminutions, visual field defects, retinal detachment etc.; 2) diagnosis confirmed by post-operative pathology. Exclusion criteria: 1) other diseases of the eyes; 2) negative results of post-operative pathology. The study was approved by the ethics committee of the Health Science Center of Yangtze University (approval number: YZLL2020-019). All 10 participants gave written informed consent prior to UVM tissue sampling.

2.2. CIB1 Gene Expression and Mutations Analysis

UALCAN is an interactive web resource for analyzing cancer transcriptome data. To analyse the expression of CIB1 across TCGA tumors, the online software tools UALCAN (http://ualcan.path.uab.edu/analysis.html) and Gene Expression Profiling Interactive Analysis 2 (GEPIA2, http://gepia2.cancer-pku.cn/#index) were used. The expression level of CIB1 in patients with UVM are generated by both UALCAN and GEPIA2, and the CIB1 expression level in UVM based on individual cancer stage, tumor histology, and gender, weight, age of the patients were analyzed using UALCAN online tool. To verify the two isoforms structural of CIB1 and compare the two isoforms usage in patients with UVM, GEPIA2 platform was performed. X: cancers, Y: isoforms, datasets from UVM. The cBioPortal for Cancer Genomics (http://www.cbioportal.org/) provides a web resource for exploring, visualizing, and analyzing multidimensional cancer genomics data [21]. In the present study, cBioPortal for Cancer Genomic was used to further detect the CIB1 gene expression and mutations in UVM.

2.3. Survival Analysis of Patients with UVM

To detect the effect of CIB1 expression level on UVM patient survival, UALCAN web-portal was used to generate the survival plot. In order to assess the combined survival effect of CIB1 expression and clinical parameters such as the body

weight, sex, race of the patient, multivariate Kaplan-Meier (KM) survival analysis was applied. R scripts were written to divide all patients into these six categories and to generate KM plot. The *P* value obtained from log-rank test was used to indicate statistical significance of survival correlation between groups. The overall survival (OS) and disease free survival (DFS), based on the expression status of CIB1 gene in patients with UVM was generated using GEPIA2. GEPIA2 uses Mantel-Cox test for the hypothesis evaluation. The cox proportional hazard ratio and the 95% confidence interval information can be included in the survival plot. Half of the patients with UVM had high expression levels of CIB1 and half had low expression levels of CIB1.

2.4. Correlation Genes Analysis

To detect the correlation genes of CIB1 in UVM, correlation analysis was used. The 20 top-rank correlation genes of CIB1 expression in UVM were obtained using GEPIA2 online tool. The expression dataset is UVM tumor from TCGA. Pearson's correlation coefficient (Pearson CC) was used to screen the top 20 correlated genes that were ≥0.78. Moreover, the top 50 positive and negative correlated genes of CIB1 expression in UVM were analyzed from UALCAN dataset. If pearson CC > 0, represent genes positively correlated with CIB1 in UVM; when, pearson CC < 0, genes negatively correlated with CIB1 in UVM. Concerning top 50 positive correlated genes, pearson CC are selected more than 0.73; Negative correlated genes, pearson CC are screened less than minus 0.53. Furthermore, the 5 top-rank positively correlated genes (ORMDL2, MRPS34, MRPS11, ATOX1 and ZDHHC12) and negatively correlated genes (RPL32, RPL14, C6orf48, LTA4H and FBL) with CIB1 in UVM were also analyzed using UALCAN online tool.

2.5. Expression and Survival Heatmap Analysis

The heatmap profile of correlation genes of CIB1 expression in UVM and additional TCGA cancer types were analyzed using an interactive heatmap, and the multiple gene comparison tools in GEPIA2 was applied. The tumor data from TCGA was selected. To compare the survival contribution of top 20 positive and negative correlated genes of CIB1 expression in UVM and additional TCGA cancer types, the survival map was calculated from TCGA-tumor specimens using the Mantel-Cox test.

2.6. Promoter Methylation Analysis

To analyze the CIB1 promoter methylation levels in patients with UVM, the CIB1 promoter methylation profile based on individual cancer stage, and gender, weight, age of patients was analyzed using UALCAN online tool.

2.7. Protein-Protein Interaction (PPI) Networks and GO Enrichment Analysis

The STRING database (http://string-db.org) provides a critical assessment and

integration of PPI, including physical as well as functional associations [18]. The PPI network of CIB1 was contrasted using STRING version 10.0 online tools. GeneMANIA (http://genemania.org) is a flexible user-friendly web site for generating hypotheses concerning gene function, analyzing gene lists and prioritizing genes for functional assays [19]. GeneMANIA was used to further analyze the related genes of CIB1. GO enrichments were analyzed using Metascape tool (http://metascape.org) explored in 2019 [20].

3. Results

3.1. Elevated Expression Level of CIB1 in Patients with UVM

To determine the expression level of CIB1 in UVM, immunohistochemical (IHC) staining was used firstly. As shown in **Figure 1A**, a significant increase expression level of CIB1 was observed from UVM tissues (middle) when compared with that in normal tissues (surrounding normal choroidal melanocytes) and that in negative control groups. Socio-demographic characteristics of patients in this study suggested that the proportion of elderly patients is increasing, and the proportion of men decreased and the proportion of women increased. To detect the CIB1 expression profile in UVM based on individual cancer stage, tumor histology, and sex, weight, age of patients, UALCAN web resource was analyzed. As shown in **Figures 1B-F**, there were no significantly difference expression levels of CIB1 based on gender (**Figure 1D**) and weight (**Figure 1E**) of the patients. However, individual cancer stages (**Figure 1B**), patient's age (**Figure 1C**) and tumor histology (**Figure 1F**) might be the potential impact factor for CIB1 expression in patients with UVM. In particular, the expression level of CIB1 is significantly elevated in stage 4 when compared with that in stage 3 (**Figure 1B**, $p < 0.001$). As shown in **Figure 1C**, older patients (81 - 100 years) exhibited higher CIB1 expression level than that in young patients (21 - 40 years, $p < 0.05$). Epithelioid cells expressed more CIB1 than that in spindle cells (**Figure 1F**, $p < 0.01$).

As shown in **Figure 1G**, cBioPortal for Cancer Genomic analysis demonstrated that there are several gains, diploid and shallow deletions were seen in TCGA-UVM samples. There are two isoforms of CIB1 have been identified, CIB1-001 and CIB1-201. To verify the two structural isoforms of CIB1 and compare the occurrence of the two major isoforms in patients with UVM, GEPIA2 dataset analysis was further used. As shown in **Figures 1H-I**, two isoforms CIB1-001 and CIB1-201 consist of 191aa and 231aa, respectively. Each of them contains the EF-hands for their multiple functions. The occurrence of CIB1-001 is more frequent in patients with UVM compared with that in CIB1-201 (**Figure 1H**).

Taken together, the result revealed that CIB1 had high expression in UVM compared with that in normal tissues.

3.2. Effect of CIB1 Expression on Patient Survival

To evaluate the prognostic value of CIB1 in patients with UVM, the overall

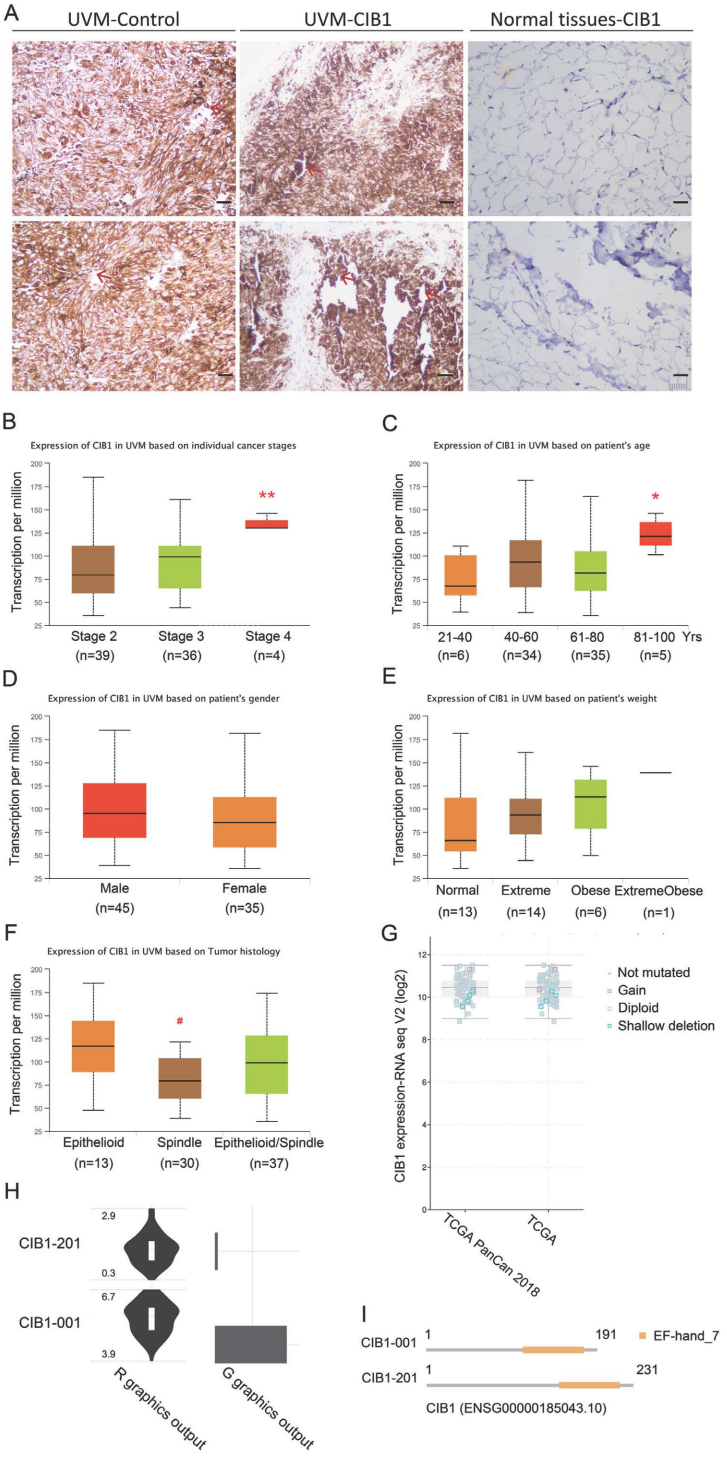

Figure 1. Expression of CIB1 in patients with UVM. A. The CIB1 expression levels in patients with UVM. Images of CIB1 immunohistochemical staining in normal tissue with CIB1 positive staining (control), UVM with CIB1 positive staining and negative staining (200x, bar = 10 μm; the lesion sites have been marked with arrows); Expression level of CIB1 in patients with UVM based on individual cancer stage (B, **$p < 0.001$, stage 4 vs stage 3), and age (C, *$p < 0.05$, 81 - 100 years vs 21 - 40 years), gender (D), weight (E) of the patients, tumor histology (F, #$p < 0.01$, epithelioid vs spindle and epithelioid/spindle vs spindle), respectively; G. The expression levels and mutations of CIB1 in patients with UVM analyzed using cBioPortal for Cancer Genomic; H. Occurrence of two CIB1 isoforms in patients with UVM. X: cancers, Y: isoforms, datasets from UVM. I. Two structural isoforms of CIB1.

survival (OS) and disease free survival (DFS) of CIB1 expression level in UVM were analyzed. GEPIA2 results demonstrated that a higher CIB1 expression level resulted in significantly lower survival probability (**Figure 2A**, OS, $p < 0.0011$ and **Figure 2B**, RFS, $p = 0.031$). UALCAN web portal analysis further exhibited the consist conclusion (**Figure 2C**, $p < 0.0001$).

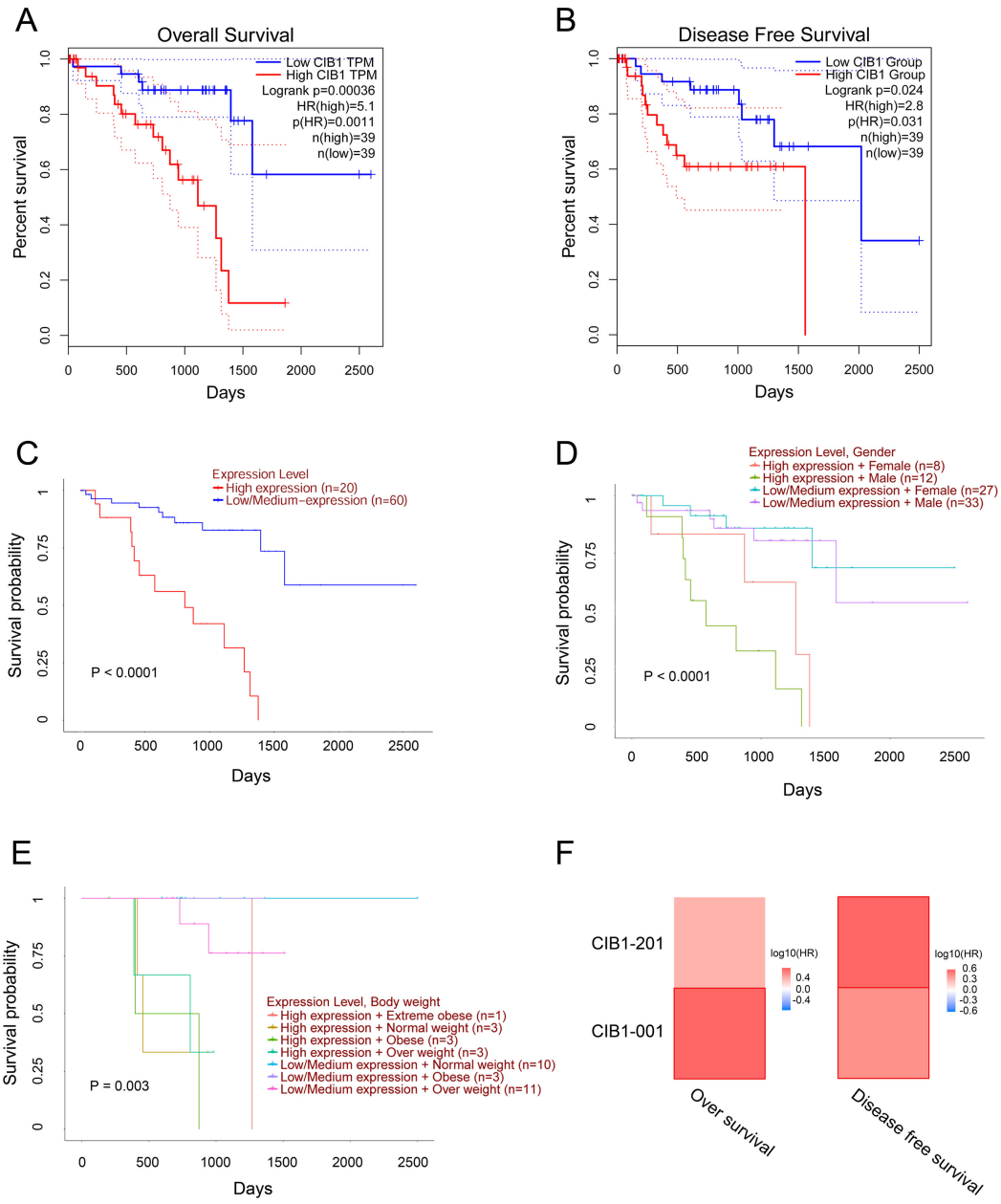

Figure 2. Effect of CIB1 expression level on survival probility in patients with UVM. A-B. A higher expression level of CIB1 was associated with poorer OS (A) and DFS (B) in patients with UVM, respectively; Median cut-off model (cut-off high and cut-off low are 50%) was selected for analysis, calculation of the hazards ratio was based on Cox PH Model. Add the 95% confidence intervals as dotted line. Axis Units are days. High groups and low groups are represented by red and blue, respectively. C. Effect of CIB1 expression levels on patient's survival was mined from UALCAN interactive web-portal; D. Effect of CIB1 expression levels & gender on UVM patient's survival; E. Effect of CIB1 expression levels & body weight on UVM patient's survival; F. Survival heatmap of two CIB1 isoforms in patients with UVM.

In addition, the survival probability was also associated with body weight and sex of the patients. As shown in Figure 2D, the male patients exhibited poorer survival probability when compared with that in female patients. Body weights of patient also effect the patient survival obviously, as shown in Figure 2E, obese and overweight patients demonstrated shorter survival time. The contribution of the CIB1 isoforms to the survival probability of patients with UVM was determined. As shown in Figure 2F, survival map analysis verified that CIB1-001 is the major isoform effect the survival of patient with UVM. These data indicated that CIB1 as a promising prognostic biomarker in patients with UVM.

3.3. Correlation Genes with CIB1 in Patients with UVM

To identify genes correlated with CIB1 in patients with UVM, the top 20 correlated genes with CIB1 expression in UVM were mined using GEPIA2 online tool and summarized in Table 1 (P CC > 0.78). Besides, the 50 top-rank positive and negative correlated genes with CIB1 expression in UVM were investigated using UALCAN tool and listed in Table 2.

Table 1. Top 50 ranked CIB1-positively and -negatively correlated genes in TCGA-UVM dataset.

Genes Number	Similar Genes	Genes ID	Pearson CC
1	ORMDL2	ENSG00000123353.9	0.85
2	MRPS34	ENSG00000074071.13	0.85
3	MRPS11	ENSG00000181991.15	0.84
4	ATOX1	ENSG00000177556.11	0.83
5	ZDHHC12	ENSG00000160446.18	0.82
6	MYL6	ENSG00000092841.18	0.81
7	POR	ENSG00000127948.13	0.80
8	MSRB1	ENSG00000198736.11	0.80
9	MPV17L2	ENSG00000254858.9	0.80
10	LAMTOR2	ENSG00000116586.11	0.80
11	DHRS7B	ENSG00000109016.17	0.80
12	ARPC1B	ENSG00000130429.12	0.80
13	PAGR1	ENSG00000238045.9	0.79
14	NDUFAF1	ENSG00000137806.8	0.79
15	GLA	ENSG00000102393.9	0.79
16	DNAJB12	ENSG00000148719.14	0.79
17	AIFM2	ENSG00000042286.14	0.79
18	ADCK5	ENSG00000173137.11	0.79
19	HM13	ENSG00000101294.16	0.78
20	ALG1	ENSG00000033011.11	0.78

Table 2. Top 20-ranked similar genes with CIB1 in patients with UVM.

Positive Correlated Genes	Pearson CC	Negative Correlated Genes	Pearson CC
ORMDL2	0.82	RPL32	−0.7
MRPS34	0.82	RPL14	−0.67
MRPS11	0.81	C6orf48	−0.66
ATOX1	0.81	LTA4H	−0.65
ZDHHC12	0.79	FBL	−0.64
C15orf63	0.79	RPL15	−0.64
NDUFAF1	0.78	RPSA	−0.63
GLA	0.78	RPL24	−0.62
ARPC1B	0.78	RPSAP58	−0.62
SEPX1	0.78	RPL3	−0.61
ROBLD3	0.78	QARS	−0.61
ADCK5	0.77	RPS3	−0.61
TNFRSF1A	0.77	CSNK2A2	−0.6
POR	0.77	RPS5	−0.6
DNAJB12	0.77	NCRNA00219	−0.59
MYL6	0.77	RPL35A	−0.59
HM13	0.77	EEF1G	−0.59
MPV17L2	0.77	RPSAP9	−0.59
DHRS7B	0.77	RPL12	−0.58
DYNLL1	0.76	LGTN	−0.58
SFXN3	0.76	SNHG7	−0.58
AIFM2	0.76	RPS4X	−0.58
S100A13	0.76	RPS13	−0.57
P4HA2	0.76	ZNF677	−0.57
SRA1	0.76	RPL10A	−0.57
WDR25	0.75	RPS18	−0.56
TCIRG1	0.75	LYRM4	−0.56
ALG1	0.75	RPL29	−0.56
CHAC1	0.75	EIF3L	−0.56
POLR3K	0.75	CTF1	−0.56
C12orf62	0.75	RPL23	−0.56
ATP6V0B	0.74	RPL38	−0.55
SIL1	0.74	MTUS1	−0.55
GSDMD	0.74	RPS2	−0.55
PFN1	0.74	RPL37	−0.55
IDH2	0.74	C14orf93	−0.55

Continued

FKBP2	0.74	RPL10	−0.55
SLC22A18	0.74	IMPDH2	−0.55
STX4	0.74	GLTSCR2	−0.55
MVP	0.74	RPS9	−0.55
IMP3	0.74	CDCA7L	−0.55
COTL1	0.74	GAS5	−0.54
DOK1	0.73	SLC25A38	−0.54
KDELR3	0.73	RPS23	−0.53
IRAK1	0.73	HNRNPA1	−0.53
NCS1	0.73	LETMD1	−0.53
MAGIX	0.73	RPL22	−0.53
MFSD5	0.73	GNB2L1	−0.53
RAB15	0.73	NMNAT3	−0.53
PSMB3	0.73	SNHG8	−0.53

As shown in **Figure 3A**, correlation analysis of the top 5 CIB1-positively correlated genes (ORMDL2, MRPS34, MRPS11, ATOX1 and ZDHHC12) and CIB1-negatively correlated genes (RPL32, RPL14, C6orf48, LTA4H and FBL) were analyzed. To further identified the similarity of the top 20 correlated genes, the expression heatmap and survival heatmap were evaluated. As shown in **Figure 3B**, the positively correlated genes expressions across TCGA tumors are very similar to that in CIB1, especially in UVM cancer types. In contrast, the negatively correlated genes expressions in patients with UVM are widely divergent to that in CIB1 (**Figure 3C**). To further analysis the survival heatmaps, as shown in **Figure 3D**, the results confirmed that the top-20 positively correlated gene showed high hazards ratio in UVM, but not in additional cancers. The top-20 negatively correlated gene showed low hazards ratio in UVM (blue) when compared with that in additional cancer types (**Figure 3E**).

3.4. Promoter Methylation Levels of CIB1 in Patients with UVM

Promoter methylation usually consider as a marker of gene inactivation [22]. To investigate the CIB1 promoter methylation profile based on individual cancer stage, and gender, weight and age of the patients, UALCAN online tool was used. As shown in **Figure 4**, the results revealed that the individual cancer stage (A), and age (C), gender (D) of the patients might not contribute greatly to the CIB1 promoter methylation in patients with UVM. However, patient's weight could alter the promoter methylation level of CIB1 in patients with UVM (**Figure 4B**, normal vs obese, $p < 0.05$).

3.5. PPI Network and GO Enrichment Analysis of CIB1

The functional interactions between proteins can provide important information

of the molecular mechanism involved. PPI network of CIB1 was presented using the STRING database (**Figure 5A**). The result indicated that CIB1 has interactions with 11 functional partners, including GUF1, CRY2, PLK3, ACTN4, PSEN2, ITGA2B and ITGB3, MAP3K5, CABP1, SPICE1, and ARHGEF1. To be specific, translation factor GUF1 [23], which promotes mitochondrial protein synthesis, and CRY2, transcriptional repressor which forms a core component of the circadian clock [24].

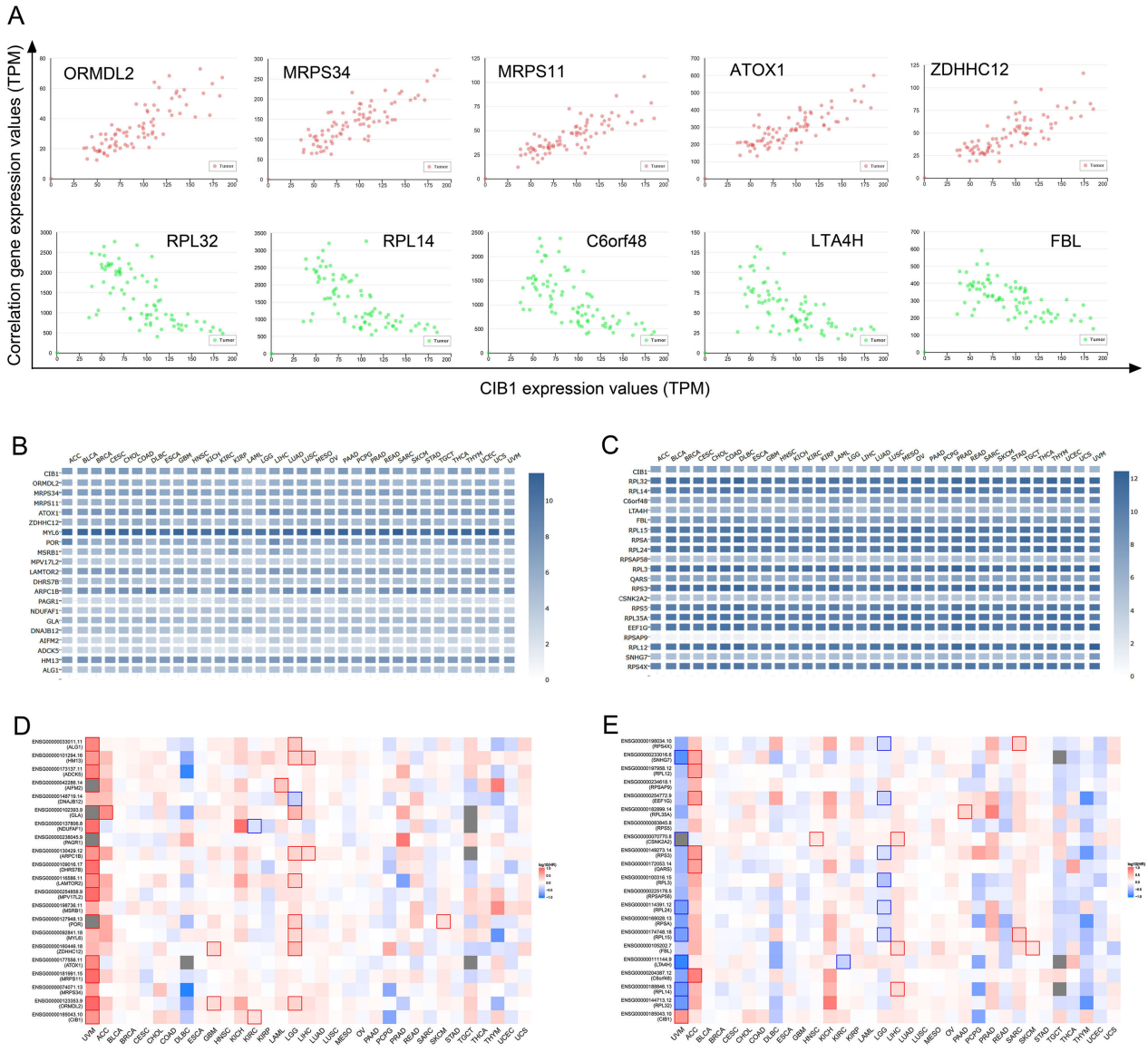

Figure 3. Correlation genes with CIB1 in patients with UVM. A. The correlations between the top 5 positively associated genes (ORMDL2, MRPS34, MRPS11, ATOX1 and ZDHHC12) and top 5 negatively correlation genes (RPL32, RPL14, C6orf48, LTA4H and FBL) with CIB1 in patients with UVM; B. Expression heatmap of 20 top-rank CIB1 positively correlated genes and CIB1 in patients with UVM and additional TCGA tumors; C. Expression heatmap of top 20 CIB1 negatively correlated genes and CIB1 gene in patients with UVM and additional TCGA tumors; D. Survival heatmap of top 20 CIB1 positively correlated genes and CIB1 gene n patients with UVM and additional TCGA tumors; E. Survival heatmap of top 20 CIB1 negatively correlated genes and CIB1 gene in patients with UVM and additional TCGA tumors.

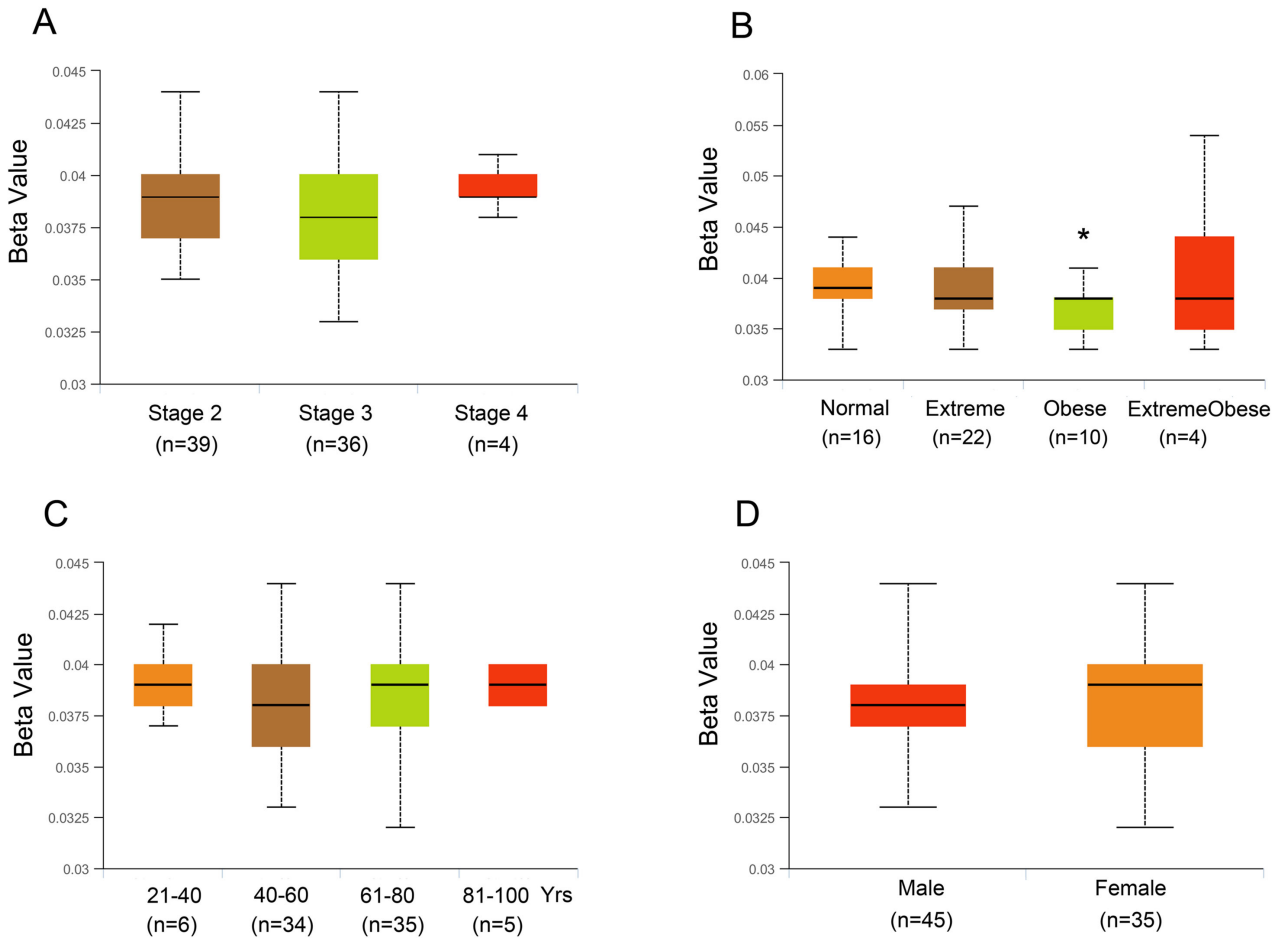

Figure 4. Promoter methylation levels of CIB1 in patients with UVM. Promoter methylation level of CIB1 in patients with UVM based on individual cancer stages (A), and weight (B), age (C), gender of the patient (D) (*$p < 0.05$, normal vs obese).

As shown in Figure 5B, 20 interactive genes of CIB1 were further identified using GeneMANIA, including PSEN2, PLK3, ITGA2B, and ITGB3, which consistent with the aforementioned correlated CIB1 genes from Figure 5A. Moreover, 16 other correlated genes from GeneMANIA dataset are PAX3, RAC3, PLK2, PSEN1, POLG, IDH2, FEZ1, ATP6V0E1, CD151, LRP10, GPX4, UBR5, POLD4, S100A10, CSTB, and C21orf33. Consistent with the aforementioned correlation analysis in Figure 3, IDH2 is just belonging to the top 50 ranked CIB1 positively correlated genes in patients with UVM.

To determine the potential function CIB1 in UVM, GO enrichment analysis of CIB1 and the genes it interacts with were performed using Metascape tool (Figure 5C and Figure 5D). The results suggested that CIB1 and the genes it interacts with are significantly enriched in ITGA2B-ITGB3-CIB1 complex, regulation of intracellular protein delivery, regulation of ion transport, cellular response to tumor necrosis factor and positive regulation of apoptotic process.

4. Discussion

Patients with UVM at the highest risk for progression to metastatic disease [25],

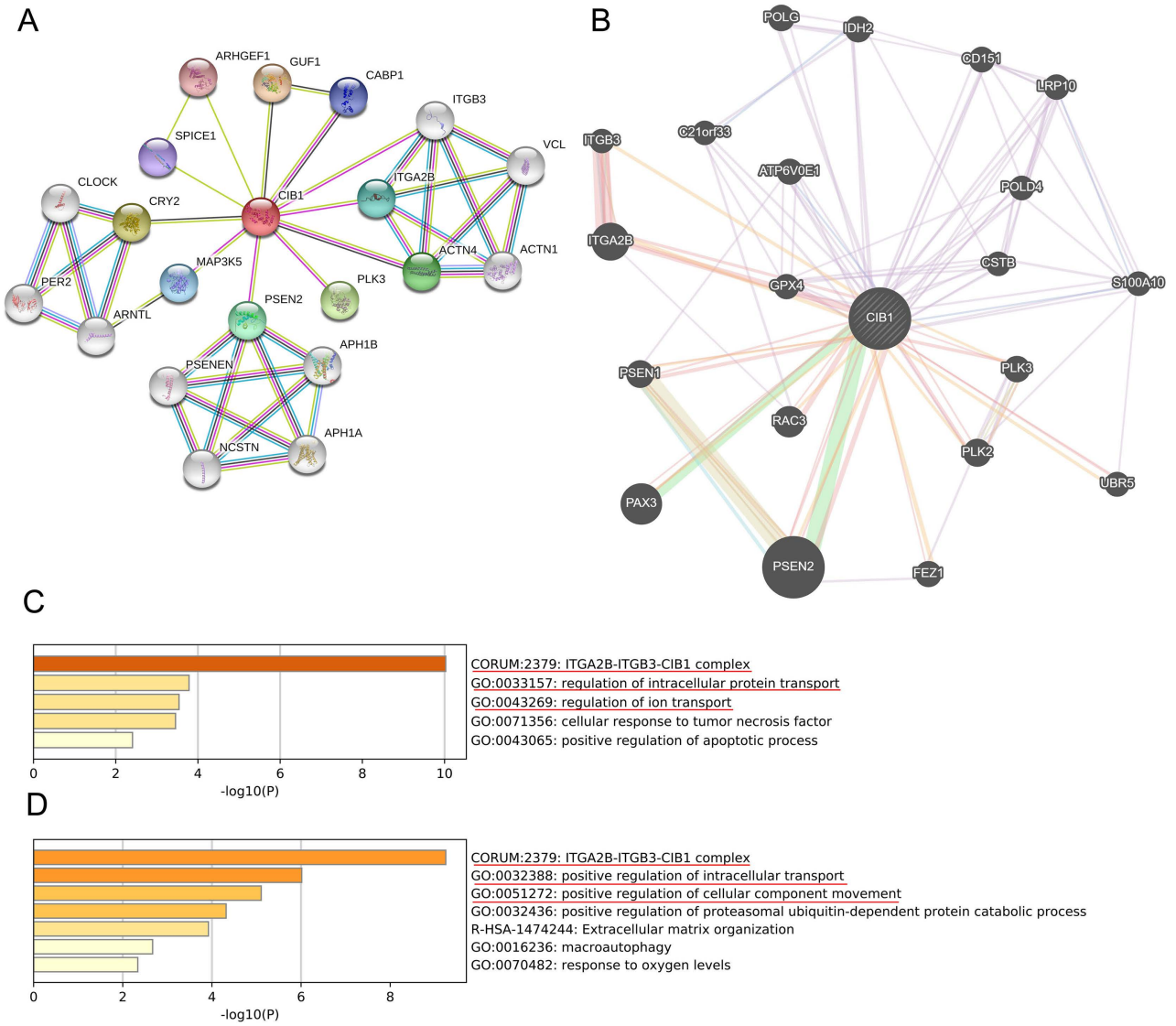

Figure 5. PPI networks and GO enrichment analysis of CIB1. A. PPI network of CIB1 and its interactive genes were constructed. Network nodes represent proteins; colored nodes represent CIB1 and the first shell of interacting proteins; white nodes represent the second shell of interacting proteins; B. PPI network of CIB1 was created using GeneMANIA. A total of 20-associated genes, with 21 total genes and 134 total links are shown. Interactions with CIB1 are indicated using stripes. C. Bar graph of enriched terms across input CIB1 and its interactive genes from Figure A, colored by p-values. Top 5 clusters with their representative enriched terms (one per cluster) were showed. Log10 (P) is the p-value in log base 10. D. GO enrichment analysis of CIB1 and the top 20 interactive genes obtained from Figure B, colored by p-values. Top 7 clusters with their representative enriched terms (one per cluster) were showed.

[26] often results in high rates of mortality; effective prognostic indicators used to evaluate the survival of patients with UVM are limited [27], [28]. In the present study, the most important discovery is reported that CIB1 as a potential diagnostic and prognostic biomarker in patients with UVM and might shed light on the UVM treatment by targeting CIB1. Our novel findings suggest that elevated CIB1 expression level in UVM compared with that in normal tissues, and several gain, diploid and shallow deletion were seen in TCGA samples. The results might assist us to understand the underlying carcinogenesis or progression of UVM better.

Moreover, the higher CIB1 transcripts per million (TPM) resulted in a significantly worse RFS and OS. The expression level of CIB1 in patients with UVM was obviously associated to body weight and gender of the patients. Besides, female gender and overweight body of the patients are considered as the important factors for facilitating their poorer survival probability. Most individuals who are obese harbor inflamed adipose tissue, which resembles chronically injured tissue, with immune cell infiltration and remodeling. Within this tumor microenvironment, several pathophysiologic changes are revealed that might contribute to cancers [29]. The positively correlated genes almost exhibited the similar expression and survival profiles to that of CIB1 in patients with UVM. The genes might have been implicated in poor prognosis and recurrence of patients with UVM. To be specific, it is identified that copper chaperone ATOX1 is required for MAPK signaling and growth in BRAF mutation-positive melanoma [30]. The mitochondrial ribosomal protein of the small subunits 34 (MRPS34) is explored to compromise protein synthesis and influence mitochondrial dysfunction [31]. Owning to similar expression and survival heatmap with CIB1 in patients with UVM, the data might provide more clues for prognostic judgment of UVM in the future. However, MYL6, ARPC1B and HM13 genes indicated the even higher expression levels than CIB1 in UVM from the top 20 ranked CIB1 correlated genes. Intriguingly, three CIB1-negatively correlated genes, C6orf48, RPSAP58 and CSNK2A2, showed similar levels of expression to CIB1 in patients with UVM. Thus, the molecular mechanism and possible applications of the correlated genes in UVM required intensively disclosed in the further study.

From the PPI network analysis, STRING and GeneMANIA identified 11- and 20-associated genes, respectively. Several CIB1-interactive genes, such as PLK3, PSEN2, ITGA2B, PAX3, RAC3, PLK2 and FEZ1, have been welled elaborated previously [11] [32]. Four same genes PSEN2, PLK3, ITGA2B and ITGB3 were identified using both STRING and GeneMANIA. It is also an interesting question that if the genes are associated with CIB1 in patients with UVM? To be specific, isocitrate dehydrogenase 2 (IDH2), which mined by GeneMANIA, is belonging to the 50 top-ranks CIB1 positively correlated genes in patients with UVM. IDH2, an important mitochondrial metabolic enzyme involved in the tricarboxylic acid cycle, is mutated in a variety of cancers [33] [34]. Increasing evidence has described that IDH2 play crucial roles in the prognosis and treatment of acute myeloid leukemia patients [35] [36]. Therefore, IDH2 is likely to be a potential biomarker for patients with UVM by further identification. Finally, Metascape tool analysis revealed that CIB1 and its correlated genes in UVM are intimately associated with ITGA2B-ITGB3-CIB1 complex, regulation of intracellular protein transport and regulation of ion transport. ITGA2B-ITGB3 receptor complex (αIIbβ3) is the paradigmatic integrin receptor. The binding of CIB1 to αIIbβ3 is verified to hamper phospholipase C (PLC)/IP3 signaling and intracellular Ca^{2+} release from ER store by IP_3R channel [11]. Combing with the GO enrichment analysis, we hypothesized that the pathogenic mechanism of CIB1 in UVM probably associated with αIIbβ3-mediated cell migration and/or intracel-

lular Ca^{2+} signaling transduction. However, further studies are required to intensively unveil the molecular mechanism and implication of CIB1 or correlated genes in UVM tumorigenesis and treatment through animal experiments or even cell experiments rather than the biogenic analysis. Moreover, the study had a pretty limited sample size, so more work needs to be done to verify and confirm the results.

5. Conclusion

In summary, the present study has identified that CIB1 is highly expressed in patients with UVM and elevated expression level of CIB1 is negatively correlated with survival probability in patients with UVM for the first time. The study might aid the identification of the underlying mechanism in UVM progression, and provide the high prognostic value of CIB1 in UVM. However, the detailed molecular pathogenesis and alteration in signaling pathways of CIB1 and the correlated genes in UVM are required to investigate in the future.

Declarations

Ethics approval and consent to participate:

This study was approved by the ethics committee of Yangtze University Health Science Center (approval number: YZLL2020-019) and participants provided written informed consent.

Author Contributions

SJ and XW designed the study. X, SJ and XW drafted the manuscript. SJ, X and XW analyzed the data and filled the tables. XW and YY revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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