Identification of PBK as a hub gene and potential therapeutic target for medulloblastoma

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Abstract. Medulloblastoma (MB) is the most frequent malignant brain tumor in pediatrics. Since the current standard of care for MB consisting of surgery, cranio-spinal irradiation and chemotherapy often leads to a high morbidity rate, a number of patients suffer from long-term sequelae following treatment. Targeted therapies hold the promise of being more effective and less toxic. Therefore, the present study aimed to identify hub genes with an upregulated expression in MB and to search for potential therapeutic targets from these genes. For this purpose, gene expression profile datasets were obtained from the Gene Expression Omnibus database and processed using R 3.6.0 software to screen differentially expressed genes (DEGs) between MB samples and normal brain tissues. A total of 282 upregulated and 436 downregulated DEGs were identified. Functional enrichment analysis revealed that the upregulated DEGs were predominantly enriched in the cell cycle, DNA replication and cell division. The top 10 hub genes were identified from the protein-protein interaction network of upregulated genes, and one identified hub gene [PDZ binding kinase (PBK)] was selected for further investigation due to its possible role in the pathogenesis of MB. The aberrant expression of PBK in MB was verified in additional independent gene expression datasets. Survival analysis demonstrated that a higher expression level of PBK was significantly associated with poorer clinical outcomes in non-Wingless MBs. Furthermore, targeting PBK with its inhibitor, HI-TOPK-032, impaired the proliferation and induced the apoptosis of two MB cell lines, with the diminished phosphorylation of downstream effectors of PBK, including ERK1/2 and Akt, and the activation of caspase-3. Hence, these results suggest that PBK may be a potential prognostic biomarker and a novel candidate of targeted therapy for MB.

Introduction

Medulloblastoma (MB) is the most prevalent pediatric central nervous system malignancy (1). The MB classification process has evolved from relying solely on histopathological features to the integration of molecular characteristics (2). The current international consensus recognizes four MB subgroups: Wingless (WNT), Sonic hedgehog (SHH), group 3 and group 4; which are based on distinctive-omic and clinical features (2,3). Somatic CTNNB1 mutations and chromosome 6 loss are common in the WNT subgroup (4), while the amplification of GLI1 or GLI2 and the deletion of Patched 1 (PTCH1) are frequently observed in the SHH subgroup (5). Aberrant MYC amplification can be detected in ~17% of patients with group 3 MB and is considered a defining feature of this subgroup (1). In addition, isochromosome 17q has been found in almost two-thirds of group 4 MB cases, and has been associated with cell division protein kinase (CDK)6 and MYCN amplification (6).

Despite considerable advances being made in the understanding of the molecular characteristics of MB, the current treatments for this disease are limited to maximal safe surgical resection, chemotherapy and cranio-spinal irradiation (1). Molecularly targeted therapy for MB remains in its infancy (7). Furthermore, although the overall survival rates have improved in recent years, a number of survivors suffer from chronic sequelae, such as neurological and neuropsychological disabilities, resulting in a poor quality of life for these children (8). In addition to the effect of the tumor itself, another main cause of these sequelae is the treatments with a high toxicity rate, particularly for patients in the very high-risk group who inevitably receive high-intensity therapeutic
regimens (1). Instead of indiscriminately acting on all rapidly dividing cells (not only cancer cells, but also certain normal cells), targeted cancer therapies focusing on interferring with specific molecules involved in oncogenesis hold the promise of being less toxic than traditional chemoradiotherapy (9). Therefore, developing more targeted therapeutic strategies for MB may prove to be instrumental in curtailing the adverse effects of conventional therapies.

In the present study, datasets comprising MB and normal brain samples from the Gene Expression Omnibus database (GEO) were analyzed and a set of hub genes with a significantly upregulated expression in MB was identified. In addition, two MB cell lines, Daoy and D341 belonging to SHH and group 3, respectively (10), were used to investigate the potential of one identified hub gene [PDZ-binding kinase (PBK)], as a therapeutic target for the treatment of MB (please see the flow chart in Fig. 1).

Materials and methods

Identification of differentially expressed genes (DEGs). RNA-seq and microarray data were retrieved from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). Microarray data comprised GSE35493 (based on Affymetrix Human Genome U133 Plus 2.0 Array, GPL570) (11) and GSE39182 (based on Agilent-014850 Whole Human Genome Microarray 4x44K G4112F, GPL6480) (12). GSE35493 included 21 MB and 9 normal brain samples. GSE39182 included 20 MB and 5 normal samples. In addition, RNA-seq data GSE148389 (based on NextSeq 550, GPL21697) contained 14 normal and 26 tumor tissues (13). All probes were annotated by annotation files. Data processing was performed using R 3.6.0 software (https://www.r-project.org/). DEGs between MB and normal samples in the GSE35493 and GSE39182 microarray datasets were screened using the Limma package (14), and GSE148389 RNA-seq data were analyzed using the edgeR package (15). An adjusted P<0.05 and |log2 fold change (FC)|≥1 were set as thresholds to identify the DEGs. Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/) were utilized to detect and present the common DEGs among the three datasets.

GO and KEGG pathway analyses of upregulated DEGs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/), which is a database which can be used for processing functional annotation with gene lists (16). The DEG results were entered to obtain the enrichment of the biological process, molecular function and cellular component terms of GO analysis and KEGG pathway terms. A P<0.05 was considered to indicate a statistically significant difference.

Protein-protein interaction (PPI) network construction and identification of hub genes. To assess the functional associations among the upregulated DEGs, the Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/) database was used to construct the PPI network of DEGs (17). Interactions with a combined score >0.4 were considered significant. The PPI network was then visualized using Cytoscape (version 3.8.0: https://cytoscape.org/) (18). Subsequently, hub genes among the upregulated DEGs were identified using the Cytoscape plugin cytoHubba. The top 10 hub genes were calculated according to the maximal clique centrality (MCC) algorithm in cytoHubba (19).

Expression analysis of the hub gene, PBK, and survival analysis. At the beginning of the present study, a number of datasets comprising MB and normal brain samples were downloaded following a search of the GEO database. GSE35493, GSE39182 and GSE148389 were selected to identify DEGs between MB and normal samples as these three datasets had relatively larger sample sizes and were based on different platforms, which was considered beneficial for obtaining more DEGs. The remaining datasets were then used as validation sets to verify the high expression level of PBK in MB, including GSE74195 (20), GSE50161 (21), GSE42656 (22), GSE19360 (23), GSE109401 (24), GSE86574 (25) and GSE62600 (26). PBK expression in the four MB subgroups was also examined in datasets GSE85217 (27), GSE37418 (28) and GSE21140 (29). To date, only a few MB datasets contain survival information, of which GSE85217 is the one with the largest cohort, and the sample size of other datasets is too small to conduct the survival analysis for four subgroups. Therefore, the prognostic values of PBK were tested in GSE85217 with the clinical data of a large cohort of patients with MB (27). Survival curves were drawn using Graphpad Prism 9 (GraphPad Software, Inc.). Survival analysis was completed using the Kaplan-Meier method and overall survival was analyzed using the log-rank test.

**MB cell lines and cell culture.** The Daoy (HTB-186) and D341 (HTB-187) MB cell lines were obtained from the American Type Culture Collection (ATCC). Daoy cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; cat. no. C11995, Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; cat. no. 10270-106, Gibco; Thermo Fisher Scientific, Inc.), 1% penicillin/streptomycin (P/S; cat. no. 15140-122, Gibco; Thermo Fisher Scientific, Inc.) and 1% non-essential amino acid (NEAA; cat. no. 11140-035, Gibco; Thermo Fisher Scientific, Inc.). D341 cells were cultured in DMEM (cat. no. C11995, Gibco; Thermo Fisher Scientific, Inc.) supplemented with 20% FBS, 1% P/S and 1% NEAA. Both cell lines were maintained under a 95% O2 and 5% CO2 humidified atmosphere in an incubator at 37˚C.

Western blot analysis. MB cells were plated at a density of 8.5x10^3 cells in 100-mm cell culture dishes and harvested following treatment with HI-TOPK-032 for 48 h. Daoy cells were treated at 0 (vehicle, DMSO), 1, 2 or 4 µM and D341 cells were treated at 0, 1 or 2 µM. Cells were lysed using RIPA lysis buffer (cat. no. P0013B; Beyotime Institute of Biotechnology), and the protein concentrations were determined using the BCA kit (cat. no. P0012; Beyotime Institute of Biotechnology). Proteins were then separated using SDS-PAGE (10% gel for PBK, ERK1/2, Akt and β-tubulin; 12% gel for cleaved caspase-3 and GAPDH) and transferred to PVDF membranes.
Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted using TRIzol® reagent (cat. no. 15596026, Thermo Fisher Scientific, Inc.) and the RNA concentrations were assayed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). cDNA was synthesized using reverse transcription kits (FastKing gDNA Dispelling RT SuperMix; cat. no. KR118, Tiangen Pharmaceutical Technology Co., Ltd.) according to the manufacturer's thermocycler guidelines (the reaction setup comprised of 4 µl of 5X FastKing-RT SuperMix and 1 µg of total RNA, and the final volume was made up to 20 µl with RNase-Free ddH2O; temperature protocol: 15 min at 95˚C for enzyme inactivation). The sequences of the PCR primer pairs were as follows: PBK forward, CCAACATTGTTGGATTATCGTG; reverse, GGCTGGCTTTATATCGTTCTCT; and actin beta (ACTB) forward, CATGTACGTGCCATCCAGGC; reverse, CTCTTATTAGTCCAGCACTG. qPCR was then performed according to the manufacturer's instructions [one cycle of initial denaturation at 95˚C for 3 min, 40 cycles of PCR (5 sec at 95˚C for denaturation and 1 sec at 60˚C for annealing/extension), and ended with a melting/dissociation curve stage (15 sec at 95˚C, 1 min at 60˚C and 1 sec at 95˚C)] at a final volume of 20 µl/well using SYBR-Green Talent qPCR Premix (10 µl; cat. no. FP209, Tiangen Biotech, Co., Ltd.), forward and reverse primers (0.6 µl, Sangon Biotech, Co., Ltd.), cDNA (1 µl/50 ng). ROX Reference Dye (0.4 µl, Tiangen Biotech, Co., Ltd.) and RNase-Free ddH2O (7.4 µl, Tiangen Biotech, Co., Ltd.). The reaction was performed on the Applied Biosystems QuantStudio 3 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). In each run, the expression levels of PBK were normalized to those of ACTB as a housekeeping gene [i.e., the expression level of ACTB was set as 1; the ΔCq value was calculated as follows: ΔΔCq=Cq(PBK)-Cq(ACTB); the difference in the expression level between PBK and ACTB was 2ΔΔCq-fold and the expression level of PBK was then normalized as 1/2ΔΔCq].

Cytotoxicity and cell proliferation assay. The Cell Counting Kit-8 (CCK-8; cat. no. G10001, Glpbio Technology Inc.) assay was used to detect the viability of the control and HI-TOPK-032-treated cells. The Daoy and D341 cells were seeded in 96-well plates at a density of 2,000 and 10,000 cells per well in 100 µl of culture medium, respectively. For the cytotoxicity assays, the cells were treated with HI-TOPK-032 at 0 (vehicle, DMSO), 0.5, 1, 1.5, 2, 2.5 and 3 µM. Following treatment for 24 h (Daoy cells) or 72 h (D341 cells), 10 µl of CCK-8 solution was added to each well followed by incubation for 3 h. The absorbance values were measured using an ELX808 microplate reader (BioTek Instruments, Inc.) at a wavelength of 450 nm. Cell viability was calculated using the absorbance value of the treated groups divided by the absorbance value of the control groups and multiplied by 100%. Sigmoidal dose-response curves were fitted using non-linear regression in Graphpad Prism 9 (Graphpad Software, Inc.) to determine the IC50 values. For the cell proliferation assay, the absorbance values were measured following treatment with HI-TOPK-032 at 0, 1 or 2 µM for 0, 24, 48, or 72 h.

Apoptosis assay. The MB cells were seeded at a density of 1x104 cells in 60-mm cell culture dishes and treated with HI-TOPK-032 at 0, 2 (D341 cells) or 4 µM (Daoy cells) for 24 h. The cells were then collected and washed twice with PBS followed by staining with Annexin V-APC/7-AAD (Apoptosis Detection kit; cat. no. KGA1017, Nanjing KeyGen Biotech Co., Ltd.) and Hoechst 33342 (cat. no. C1027, Beyotime Institute of Biotechnology) according to the manufacturer’s protocol. Images were obtained using an ECLIPSE Ti2-E inverted microscope (Nikon Corporation) and the percentage of EdU-positive cells was quantified using ImageJ software (Version 1.53m; National Institutes of Health).

Statistical analysis. Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software Inc.). All experiments were repeated three times. Quantitative results are presented as the mean ± standard deviation (SD). Statistically significant differences were assessed using an unpaired Student’s t-test, one-way or two-way ANOVA with Tukey’s HSD post hoc test. A value of P<0.05 was considered to indicate a statistically significant difference.
Results

Identification of upregulated hub genes in MB. The present study first screened for genes differentially expressed between MB and normal brain samples to identify potential therapeutic targets in MB. Following RNA-seq and microarray data analyses, differential mRNA expression between MB and normal samples in three datasets was found (Fig. 2A). In GSE148389, 6,906 DEGs were identified (3,216 upregulated and 3,690 downregulated genes). In GSE35493, 5,568 DEGs were determined (2,793 upregulated and 2,775 downregulated genes). In GSE39182, 2,405 DEGs were detected (818 upregulated and 1,587 downregulated genes). Venn diagrams presented the overlapping DEGs in the three datasets. A total of 282 upregulated and 436 downregulated genes were identified as common between the three datasets (Fig. 2B).

Subsequently, the upregulated DEGs were submitted to DAVID to examine their functions and potential roles in the molecular tumorigenesis of MB. Following the enrichment analysis, the top 10 significant terms of GO analysis in three categories (biological process, molecular function and cellular component) and the significant KEGG enrichment terms were screened. In biological process, the upregulated genes were associated predominately with cell division, mitotic nuclear division, DNA replication and the G2/M transition of mitosis (Fig. 2C). In molecular function, the upregulated genes were involved primarily in protein binding, DNA binding and ATP binding (Fig. 2D). In addition, the cytosol, cytoplasm, nucleus and nucleoplasm were significantly associated with upregulated genes in the cellular component (Fig. 2E). The KEGG pathway analysis revealed that upregulated genes were enriched, particularly in DNA replication and the cell cycle (Fig. 2F).

To further investigate the functional associations among the upregulated DEGs, the STRING online database was utilized to analyze the PPI network of these genes. After removing isolated and partially connected nodes, a grid network was constructed using Cytoscape software (Fig. 2G). The Cytoscape plugin cytoHubba was then employed to determine the hub genes in the PPI network. Finally, the top 10 hub genes [cell division cycle 20 (CDC20), kinesin family member 2C (KIF2C), nucleolar and spindle associated protein 1 (NUSAPI), PBK, TTK protein kinase (TTK), kinesin family member 20A (KIF20A), DNA topoisomerase II alpha (TOP2A), CDK1, assembly factor for spindle microtubules (ASPM) and Aurora kinase A (AURKA)] were determined using the MCC algorithm in cytoHubba (Fig. 2G).

Validation of the high expression and prognostic significance of the hub gene, PBK, in MB. According to the GO and KEGG pathway analysis, the upregulated genes were predominantly enriched in the cell cycle, DNA replication and cell division. Among the top 10 hub genes, PBK encodes PDZ-binding kinase, also known as T-lymphokine-activated killer (T-LAK) cell-originated protein kinase (TOPK), which is a serine/threonine protein kinase belonging to the mitogen-activated protein kinase kinase (MAPKK) family and plays a vital role in mitotic progression (30,31). Furthermore, researchers have previously reported that PBK is highly expressed in cerebellar granule cell precursors of early postnatal mice and functions as a crucial regulator of progenitor proliferation and self-renewal (32). Thus, given that MBs are embryonal tumors originating from stem cells or progenitor cells in the cerebellum or posterior fossa (33), the present study focused on the possible role of PBK in MB. The significant upregulation of PBK was first verified in MB by interrogating and analyzing additional independent gene expression datasets, including data from patients with MB (GSE74195, GSE50161 and GSE42656) and a spontaneous mouse model of MB (GSE19360) (Fig. 3A-D).

Since MB is currently divided into four subgroups, the expression level of PBK was also examined in the different subgroups. Of note, PBK was highly expressed in all MB subgroups compared with normal brain samples (Fig. 3E-G), suggesting that PBK may be involved in the tumorigenesis of all subgroups. This is consistent with the function of PBK as a mitotic serine/threonine kinase and the rapid proliferating rate of MB cells. In addition, PBK expression varied among the MB subgroups, with group 4 MBs appearing to have the lowest expression level compared to the other subgroups (Fig. 3H-J). Subsequently, the prognostic significance of PBK in MB was assessed by analyzing the survival information of patients with MB from a large cohort. The overall survival curves revealed that a high PBK expression was significantly associated with poorer clinical outcomes in SHH, group 3 and group 4 MBs, apart from WNT MB (Fig. 4). Taken together, these results indicate that PBK is a crucial upregulated hub gene in MB and is likely to serve as a prognostic marker.

Targeting PBK inhibits the proliferation of MB cells and reduces the phosphorylation of downstream signaling molecules. To further examine the potential of PBK as a therapeutic target for MB, two commonly used MB cell lines, Daoy and D341 belonging to the SHH and group 3 respectively, were employed to perform experiments in vitro. First, PBK expression was detected in Daoy and D341 cells using RT-qPCR and western blot analysis. The results revealed that both cell lines...
had a robust expression of PBK (Fig. 5A and B). While the D341 cells exhibited a higher PBK mRNA expression, the Daoy cells appeared to have a higher protein expression, suggesting that the translation efficiency of PBK may differ between the two cell lines. However, this inconsistency may also be caused by the different reference genes (housekeeping genes) used in the two assays. The MB cells were then treated with a widely-used PBK inhibitor (HI-TOPK-032) to observe its effects on cell viability.
The viability of the Daoy and D341 cells was effectively inhibited by HI-TOPK-032 in a concentration dependent manner with an IC50 value of 1.241 and 1.335 µM, respectively (Fig. 5C and D). Moreover, HI-TOPK-032 also markedly decreased MB cell growth in a manner dependent on the degree of PBK suppression, as shown in Fig. 5E and F. Consistently, the two MB cell lines treated with HI-TOPK-032 at a concentration of 1 or 2 µM exhibited a lower proliferation rate than the control group in the EdU assay (Fig. 5G). These data demonstrated that targeting PBK with its specific inhibitor significantly impaired the proliferation of MB cells in vitro.

The present study then examined the phosphorylation status of two critical downstream targets of PBK to elucidate the mechanisms underlying the effects of HI-TOPK-032 on MB cell proliferation. Western blot analysis revealed that treatment with the PBK inhibitor, HI-TOPK-032, resulted in a slight or moderate reduction in the total levels of downstream signaling molecules, including ERK1/2 and Akt (Fig. 5H). However, a considerable decrement in the phosphorylated form of downstream proteins was observed in the HI-TOPK-032-treated MB cells (Fig. 5H). These two signaling molecules have been reported as essential downstream effectors of PBK in regulating cell proliferation in MBs.
proliferation (34,35). Additionally, the level of PBK in the MB cells remained stable following HI-TOPK-032 treatment (Fig. 5H), in accordance with previous studies (36,37). Taken together, these results suggest that HI-TOPK-032 may inhibit the proliferation of MB cells by suppressing the phosphorylation of downstream target proteins of PBK by blocking its kinase activity.

PBK inhibitor HI-TOPK-032 promotes the apoptosis of MB cells with the activation of caspase-3. To examine the effects of PBK inhibitor specifically on cell survival, cell apoptosis was measured by monitoring the externalization of phosphatidylserine (PS) in MB cells treated with HI-TOPK-032. Since HI-TOPK-032 would remain in the cell pellets following centrifugation and its color would interfere with the accuracy of flow cytometry detection, Annexin V/7-AAD-positive cells were observed and quantified under a fluorescence microscope. Annexin V single-positive cells represent early apoptotic cells, while Annexin V and 7-AAD double-positive cells indicate late-stage apoptotic cells and necrotic cells. The percentage of Annexin V single-positive cells was markedly higher in the HI-TOPK-032-treated group as compared with the control group, and the proportion of double-positive cells also exhibited similar results (Figs. 6A and B, and 7A and B). Subsequently, the activation of caspase-3 was examined using western blot analysis to further examine the apoptosis of MB cells. In line with PS externalization, the level of cleaved caspase-3 in the Daoy and D341 cells was substantially increased following treatment with HI-TOPK-032 (Figs. 6C and 7C). Thus, targeting PBK also induces the caspase-dependent apoptosis of MB cells.

**Discussion**

As the most frequent malignant brain tumor in pediatrics, MB not only poses a grave threat to the lives of children, but also leads to disabling consequences and a poor quality of life for survivors. As the standard therapeutic regimens for MB (surgical intervention followed by cranio-spinal irradiation and adjuvant chemotherapy) often lead to a high morbidity rate, numerous patients suffer from short- and long-term sequelae following treatment (1). Targeted therapies provide further options for managing this refractory disease and possibilities to reduce the treatment-related toxicity (7). The present study first identified the DEGs between MB and normal brain samples, and then screened 10 hub genes from the significantly upregulated genes in MB. These hub genes have been reported in multiple cancers (e.g., breast, prostate cancer and glioma) by their oncogenic functions (38−47); however, but to the best of our knowledge, PBK has not been previously studied as a therapeutic target for MB. Therefore, PBK was selected as the target gene for further exploration in the present study.

PBK, a mitotic serine/threonine protein kinase belonging to the MAPKK family, has been found to be expressed exclusively in proliferating and multipotent cells, particularly in germinal and fetal cells, as well as cancer cells (35). Additionally, Dougherty et al (32) reported PBK expression in rapidly proliferating central nervous system cells of mice, such as adult subependymal neuronal progenitors and granular cell precursors of the postnatal cerebellum, and its suppression in neurons, mature glia and quiescent cells. MB is a type of embryonal tumor, which is now considered to arise from
stem cells or progenitor cells in the cerebellum or posterior fossa (33). Hence, the significant upregulation in PBK expression in MB suggests that this protein may play a critical role in the pathogenesis of MB. The present study then verified the highly expressed level of PBK in MB by examining several independent gene expression datasets. Notably, the expression level of PBK was markedly higher in all MB subgroups than normal brain tissue, and a high PBK expression was associated with a poor clinical outcome in SHH, group 3 and group 4 MBs.
Figure 6. Pharmacological inhibition of PBK promotes the apoptosis of Daoy cells with the activation of caspase-3. (A and B) The percentage of apoptotic cells was significantly higher in the HI-TOPK-032-treated group compared with the control group. Scale bar, 150 µm. Quantitative results are presented as the mean ± SD. Statistical significance according to an unpaired Student's t-test is indicated: *P<0.05. (C) Western blot analysis revealed that the level of cleaved caspase-3 in Daoy cells was substantially increased following treatment with HI-TOPK-032. PBK, PDZ binding kinase.

Figure 7. Targeting PBK with HI-TOPK-032 induces the apoptosis of D341 cells in vitro. (A and B) The percentage of apoptotic cells was significantly increased in the HI-TOPK-032-treated D341 cells. Scale bar, 100 µm. Quantitative results are presented as the mean ± SD. Statistical significance according to an unpaired Student's t-test is indicated: *P<0.05. (C) Western blot analysis revealed that the level of cleaved caspase-3 in D341 cells was substantially increased following treatment with HI-TOPK-032.
Currently, investigations on targeted therapies for MB are mainly based on distinctive aberrant signaling pathways in a specific subgroup, such as the hedgehog pathway in the SHH subgroup, or more general alterations of certain pathways in MB, such as the PI3K pathway (6,7). Since WNT MBs have a favorable prognosis, major efforts have been made to search for novel therapeutic approaches for non-WNT MBs. Interfering with the PTCH1/SMO/SUFU/GLI axis in the hedgehog pathway with small molecules is considered a promising therapy for SHH MBs. The SMO inhibitor, vismodegib, for instance, is being/has been tested in some clinical trials (NCT01878617) (48). However, resistance to search for novel therapeutic approaches for non-WNT MBs also influences the outcome in non-WNT MBs. Moreover, targeting PBK with its inhibitor impaired the proliferation and induced the apoptosis of MB cells on tumor growth in vivo by using animal models, particularly orthotopic xenograft models. The preclinical testing of PBK inhibitors in animal models of MB, including patient-derived orthotopic xenograft models, is also an essential step in confirming the efficacy of PBK-targeted therapy.

In conclusion, the present study identified PBK as a hub gene with an upregulated expression in MB. The aberrant expression of PBK was validated in all MB subgroups and higher expression levels of PBK also indicated poorer clinical outcomes in non-WNT MBs. Moreover, targeting PBK with its inhibitor impaired the proliferation and induced the apoptosis of two MB cell lines in vitro. Thus, PBK may prove to be a potential prognostic biomarker and therapeutic target in the management of MB.

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Availability of data and materials

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ML, HW, YD and WZ conceived the study and wrote the manuscript. HW and ML performed the bioinformatics analyses. YD and ML conducted the experiments and analyzed the data. HY, ZZ, QH, YB, PW, MZ and JG made substantial contributions to the design of the study. All authors discussed the results and revised the manuscript. YD, HW, ML and WZ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.
Competing interests

The authors declare that they have no competing interests.

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