

Short Communication

A novel mutation in SMARCB1 associated with adult Coffin-Siris syndrome and meningioma

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Abstract

SMARCB1 encodes a core subunit of the SWI/SNF chromatin remodeling complex, which plays a crucial role in the regulation of gene expression. Germline mutations in the *SMARCB1* gene have been linked to early childhood Coffin-Siris syndrome type 3 (CSS3), a rare congenital malformation syndrome characterized by severe developmental delay and intellectual disability. In this study, we report a family of two adult CSS3 patients with a novel missense *SMARCB1* mutation (c.1091A>C, p.Lys364Thr) identified through whole-exome sequencing (WES). Both patients exhibit selective difficulties in verbal learning and experience language delays. Additionally, the development of meningioma is confirmed in one of the patients. Mechanistic studies suggest that this missense mutation may abnormally activate the MAPK signaling pathway, which is implicated in the pathogenesis of tumor progression and neurodevelopmental disorders. This is the first reported case of a germline mutation in the *SMARCB1* gene associated with both CSS3 and meningioma, thereby expanding the phenotypic spectrum of *SMARCB1*-related disorders.

Key words *SMARCB1*, SWI/SNF, neurodevelopmental disorder, meningioma

Introduction

The mammalian SWI/SNF (Switch/sucrose non-fermentable) complex is an evolutionarily conserved, ATP-dependent chromatin remodeling complex that functions as an epigenetic modulator in the regulation of gene accessibility and expression [1–3]. Germline variants in genes encoding the core subunits of the SWI/SNF complex, including *SMARCB1*, *SMARCA4*, *SMARCA2*, *SMARCE1*, *ARID1A*, and *ARID1B*, have been implicated in neurodevelopmental disorders [4–8]. One such disorder is Coffin-Siris syndrome (CSS), a rare intellectual disability syndrome characterized by developmental delay, hypotonia, coarse facial features, feeding difficulties in infancy, and fifth digit anomalies [7,9–12]. Notably, mutations in *SMARCB1* (also known as SNF5, BAF47, and INI1) are often associated with individuals exhibiting more severe intellectual disability and choroid plexus hyperplasia, a condition referred to as CSS3 (OMIM# 614608) [4,13,14]. In addition, genome-sequencing

studies have revealed the extensive role of the SWI/SNF complex in cancer, with approximately 25% of all human tumors displaying aberrations in these genes [15–17]. *SMARCB1* is recognized as a tumor suppressor, and its mutations have been linked to malignant rhabdoid tumors, schwannomas, and meningiomas, either individually or concurrently [18,19]. *SMARCB1* consists of 385 amino acids, which include the N-terminal winged-helix DNA binding domain [20], two tandem repeat domains, and the highly conserved putative coiled-coil C-terminal domain (CTD) [14]. Notably, single-residue mutations closely associated with CSS and cancer are enriched in the CTD [14,21]. However, it remains unclear whether individuals with CSS3 concurrently develop tumors due to the limited understanding of the adult phenotypes of these patients.

In this study, we identified a novel germline missense mutation in two members of a family with CSS3, one of whom developed meningioma. This report expands the phenotypic spectrum

associated with *SMARCB1*-related disorders.

Materials and Methods

Patients

The patients in this study are a pair of siblings. According to previously described method [22], genomic DNA was extracted from peripheral blood samples and the whole-exome sequencing (WES) was performed on both siblings and their parents, comprising a total of four family members. Sanger sequencing was employed to assess the presence of candidate pathogenic variants in the family members, including the sister's two children. Additionally, the father's sperm DNA was analyzed to investigate potential germline mosaicism. This study was approved by the Ethics Committee of Henan Provincial People's Hospital (Approval No. 2020-75). The parents have signed informed consent and agreed to publish the data included in this article.

Cell culture

The IOMM-Lee cell line was purchased from the American Type Culture Collection (ATCC; Manassas, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a humidified atmosphere containing 5% CO₂ at 37°C.

Overexpression of wild-type and mutant *SMARCB1*

Wild-type and A1091C-mutated *SMARCB1* overexpression plasmids were constructed by Gene Universal Technology (Hefei, China) through the cloning of the human *SMARCB1* coding sequence (Gene ID: 6598) into the lentiviral vector, followed by lentivirus packaging and validation. IOMM-Lee cells were subsequently infected with wild-type or mutated lentivirus, followed by selection with 2 µg/mL puromycin (Solarbio, Beijing, China) to obtain stably expressing cells. Bright field and fluorescent images were acquired using Olympus microscope.

Western blot analysis

Western blot analysis was performed as described previously [23]. The following antibodies were used: anti-*SMARCB1* antibody (20654-1-AP, 1:1000 dilution; Proteintech, Rosemont, USA), anti-P38 MAPK antibody (#9212, 1:1000 dilution; Cell Signaling Technology, Danvers, USA), anti-phospho-P38 MAPK antibody (AF4001, 1:1000 dilution; Affinity, Cincinnati, USA), anti-ERK1/2 antibody (AF0155, 1:1000 dilution; Affinity), anti-phospho-ERK1/2 antibody (AF1015, 1:1000 dilution; Affinity), anti-ACTB antibody (66009-1-Ig, 1:3000 dilution; Proteintech), and HRP-conjugated goat anti-rabbit antibody (SA00001-2, 1:10000 dilution; Proteintech). Image J was used to quantify relative protein expression.

RNA sequencing

Total RNA was extracted from IOMM-Lee cells via TRIzol (Invitrogen, Carlsbad, USA) according to Illumina standard instruction (VAHTS Universal V6 RNA-seq Library Prep Kit for Illumina®). An Agilent 4200 Bioanalyzer (Agilent Technologies, Santa Clara, USA) was used to evaluate the concentration and size distribution of the cDNA library, followed by sequencing with an Illumina Novaseq 6000 (Illumina, San Diego, USA). Raw reads were filtered and mapped to human RefSeq using Hisat2 (version: 2.0.4). Significant differential expressed genes (DEGs) were identified as

those with a False Discovery Rate (FDR) < 0.05 and a fold change > 2 via DESeq2 software. An online platform (<https://www.bioinformatics.com.cn>) was used for data analysis and visualization. The clean RNA-seq data used in this study have been deposited in the Gene Expression Omnibus (GEO) database of NCBI with the accession number GSE273963.

In-silico prediction

In silico analysis was performed to evaluate the pathogenicity of missense variants through online softwares including Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>), MutationTaster (<https://www.mutationtaster.org/>), VarSite (<https://www.ebi.ac.uk/thornton-srv/databases/VarSite>), and DynaMut2 (<https://biosig.lab.uq.edu.au/dynamut2>). The three-dimensional structure of *SMARCB1* was obtained from the PDB database (<https://www1.rcsb.org/>), with the PDB ID: 7Y8R. The online tool AlphaFold v3 [24] (<https://golgi.sandbox.google.com/>) was subsequently utilized to establish a structural model of the protein *SMARCB1_K364T*.

Cell proliferation and migration assays

To evaluate cell proliferation, growth curves for both wild-type and *SMARCB1*-mutant cells were generated via the Cell Counting Kit-8 (CCK-8) assay. Briefly, 10,000 cells were plated in 96-well plates and incubated in culture medium for 0, 24, 48, or 72 h. The optical density (OD) values were measured at 450 nm with a microplate reader. To evaluate cell migration, wound healing assay was conducted as previously described [25], and the relative width of the scratch was measured using ImageJ (NIH, Bethesda, USA).

Droplet digital PCR (ddPCR)

Genomic DNA (50 ng and 100 ng) from the father's sperm (I:1) was extracted to detect the germline mosaicism of the c.1091A>C variant via the QX200 AutoDG Droplet Digital PCR System (Bio-Rad, Hercules, USA). A total of 50 ng of genomic DNA extracted from whole blood at ratios of II:1 and III:1 was used as a positive and negative control, respectively. The wild-type sequence was labeled with the FAM probe, whereas the c.1091A>C variant was labeled with the VIC probe. The ratio of VIC copies to the sum of VIC copies and FAM copies was used to identify the level of mosaicism.

Immunohistochemistry

Following the surgical procedure, tumor tissue samples were dispatched to the pathology department for thorough pathological examination and immunohistochemical staining. The samples were fixed in 3.7% neutral formaldehyde, followed by dehydration using graded alcohol, embedding in paraffin, and sectioning to a thickness of 3 µm. Immunohistochemistry was performed using the EnVision two-step method, utilizing antibodies including S100, EMA, STAT6, and SSTR2A, all obtained from Maixin Biotechnology (Fuzhou, China). The entire process was carried out on a fully automatic immunohistochemistry instrument (Leica, Heidelberg, Germany), with subsequent analysis conducted after scanning via a fully automated scanner (Leica).

Statistical analysis

Data were presented as the mean ± SD, and two-tailed Student's *t*-test was used to analyze statistical differences between two groups. *P* < 0.05 was considered statistically significant.

Results

Clinical characterization and genetic analysis of the patients

The patients, siblings born to unrelated healthy Chinese parents following full-term delivery, consisted of a 36-year-old sister and a 33-year-old brother (Figure 1A). The sister began walking and speaking at 14 months, whereas the brother achieved these milestones three months later. Neither sibling experienced feeding difficulties, significant growth retardation, or severe intellectual disability. However, both exhibit coarse facial features, including thick eyebrows, wide mouths, long eyelashes, and thick lips, along with musculoskeletal findings, particularly hypoplastic fifth toenails (Figure 1B,C). Notably, they display mild selective neuropsychological deficits, such as poor learning ability, impaired memory, slow reactions, and language delay, which differ from the severe intellectual disability observed in previously reported cases. Additionally, the brother suffered from poor eyesight, strabismus, and abnormal hearing (Figure 1D).

Trio-based whole exome sequencing (Trio-WES) identified a missense mutation (GenBank: NM_003073.5; c.1091A>C) within exon 8 of the *SMARCB1* gene, which is associated with Coffin-Siris syndrome 3 (CSS3, OMIM#614608). This mutation is not listed in the ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) or HGMD databases (<https://www.hgmd.cf.ac.uk/ac/index.php>) and lacks allele frequency data in the gnomAD database (<https://gnomad.broadinstitute.org/>). In accordance with the guidelines of the American College of Medical Genetics and Genomics (ACMG), it is classified as likely pathogenic (Figure 1E,F). Sanger sequencing confirmed the heterozygous mutation in both patients but not in their parents, indicating a *de novo* pattern (Figure 1G). Given the low probability of two siblings independently inheriting the same *de novo* mutation, we speculated germline mosaicism, which was confirmed through ddPCR testing of the father's sperm, revealing a mutation ratio of approximately 12% (Figure 1H).

Further examinations ruled out other CSS3-related developmental abnormalities in the heart, brain, renal and urinary systems. However, the sister's brain magnetic resonance images (MRI) revealed space-occupying lesions adjacent to the cerebral falx. Subsequent surgical sampling and pathological examination revealed spindle-shaped tumor cells exhibiting increased collagen deposition. Moreover, the immunohistochemistry results indicated that STAT6 was negative, whereas EMA, S100, and SSTR2A were positive. On the basis of these findings, the tumor was classified as a World Health Organization (WHO) grade I fibrous meningioma [26], which was not detected in her brother (Figure 1I,J). Genetic testing of the tumor revealed no positive results associated with meningioma. *SMARCB1* mutations can increase the risk of meningiomas, suggesting a potential link between *SMARCB1* mutations and the concurrent occurrence of CSS and meningiomas in this study.

Functional study of the c.1091A>C mutation in *SMARCB1*

To evaluate the impact of the c.1091A>C missense mutation on protein function, we first assessed its pathogenicity via various online *in silico* prediction tools, including Polyphen2, Varsite, Mutation Taster, DynaMut2, and AlphaFold. The results indicated that this mutation may be associated with disease (Figure 2A). The substitution of lysine 364 with threonine (K364T) alters the protein's hydrophobicity, increases the formation of hydrogen

bonds with adjacent amino acids, and enhances the overall interactions within the 3D structure (Figure 2B). Furthermore, lysine 364 is highly conserved across multiple mammalian species (Figure 2C).

We established a stable IOMM-Lee cell line, a meningioma cell line expressing either wild-type or K364T-mutated *SMARCB1*, through lentivirus infection and subsequent puromycin selection (Figure 2D). Transcriptome analysis revealed that the K364T mutation induced significant alterations in gene expression profiles, resulting in 22 DEGs, with $|\log_2FC| > 1$ and $FDR < 0.05$ (Figure 2E, F). KEGG enrichment analysis of the significantly up-regulated genes revealed that pathways associated with neurodegeneration and amyotrophic lateral sclerosis, which are associated with *MAPK14*, *DNAL1*, and *GPX1*, are aberrantly activated (Figure 2G). *MAPK14*, also known as P38 MAPK, is a critical protein in the MAPK signaling pathway and is involved in various biological processes [27]. Its abnormal activation has been implicated in neurodevelopmental disorders as well as tumor progression in cancers, including meningioma [28–31]. *SMARCB1* plays a crucial role in SWI/SNF complex-involved activation of enhancers and bivalent promoters [32]. We examined ChIP-seq data from the Cistrome database (<http://cistrome.org/db>) and found that *SMARCB1* deficiency significantly reduced the binding affinity of the SWI/SNF complex to the promoter region of *MAPK14* (Figure 2H). Furthermore, western blot analysis results confirmed a prominent increase in the expression of P38 MAPK, ERK1/2, and phosphorylated ERK1/2 in *SMARCB1*-mutant cells (Figure 2I). Consistently, growth curves revealed greater proliferative activity in *SMARCB1*-mutant cells than in control cells, and wound healing assays indicated that *SMARCB1* mutation enhanced cell migration (Figure 2J,K). These findings suggest that the c.1091A>C mutation in the *SMARCB1* gene may affect the transcriptional regulation function of the SWI/SNF complex, thereby activating the MAPK signaling pathway and contributing to the development of CSS and meningiomas.

Virtual screening of small molecule-targeting drugs for mutated *SMARCB1*

Having preliminarily confirmed the role of the mutated *SMARCB1* protein in the pathogenesis of CSS and meningioma within this family, we investigated whether pharmacological agents that can target the mutated *SMARCB1* protein to ameliorate the disease phenotype in affected patients exist. Accordingly, we downloaded the structures of 2726 small molecules derived from the Clinical & FDA Approved Drug Library (<https://www.apexbt.com/>) and conducted molecular docking analysis via MOE software (v2022.02). By scoring the binding conformations of small molecules with the mutated *SMARCB1*, a total of 183 small molecules exhibiting strong binding affinity were selected. Further comparison of their binding abilities with those of wild-type *SMARCB1* led to the identification of three small molecules with anti-tumor activity: the MDM2 inhibitor RG7112 [33,34], the tubulin-targeting peptide dolastatin 10 [35], and the FGFR-targeting drug erdafitinib [36]. Finally, we employed PyMOL (v2.5.4) to analyze the amino acid residues involved in the hydrogen bond interactions between *SMARCB1* and the small-molecule drugs, resulting in the creation of a 3D interaction diagram. The results indicate that RG7112 forms a hydrogen bond with lysine at position 364 of the wild-type *SMARCB1* protein, whereas its interaction with threonine at position 364 of the mutant *SMARCB1* protein is increased, leading

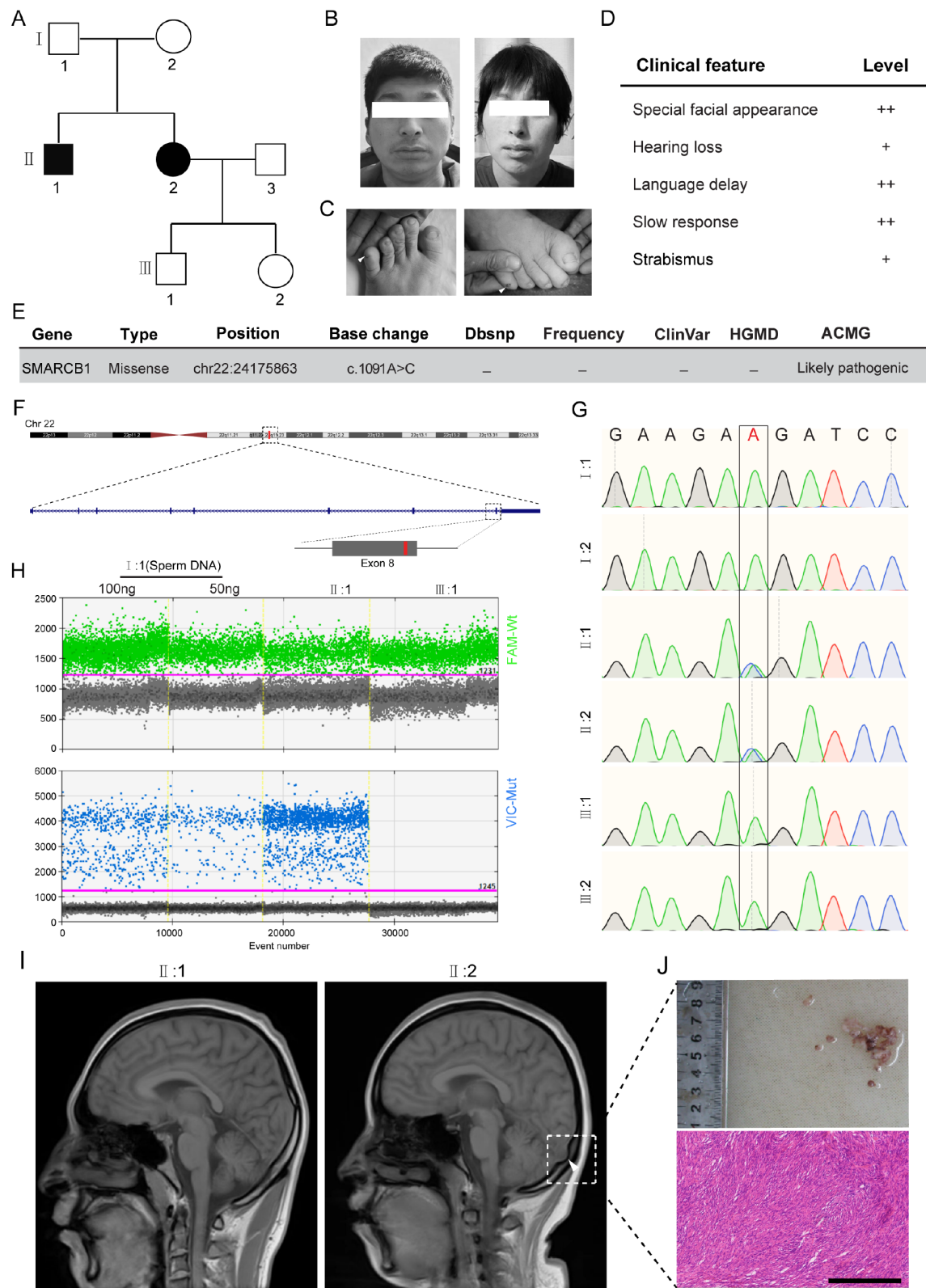


Figure 1. Genetic analysis of the *SMARCB1* variant in a Chinese family suspected of having CSS3 (A) Pedigree of the family with CSS3 in this study. The filled symbols indicate affected individuals. (B) Facial photographs of the patients. (C) Hypoplastic nail of the fifth toe. (D) Clinical phenotypes and severity observed in the patients. (E) WES analysis revealed a novel missense mutation in the *SMARCB1* gene. (F) Genomic location of the missense mutation. (G) Sanger sequencing confirmed the *de novo* heterozygous *SMARCB1* variant (c.1091A>C) in the patients (II:1 and II:2). (H) ddPCR was performed to evaluate the germline mosaicism of the sperm. (I) Representative Brain MRI of the two patients. (J) Immunohistochemical examination of meningioma in II:2 (scale bar: 100 μm).

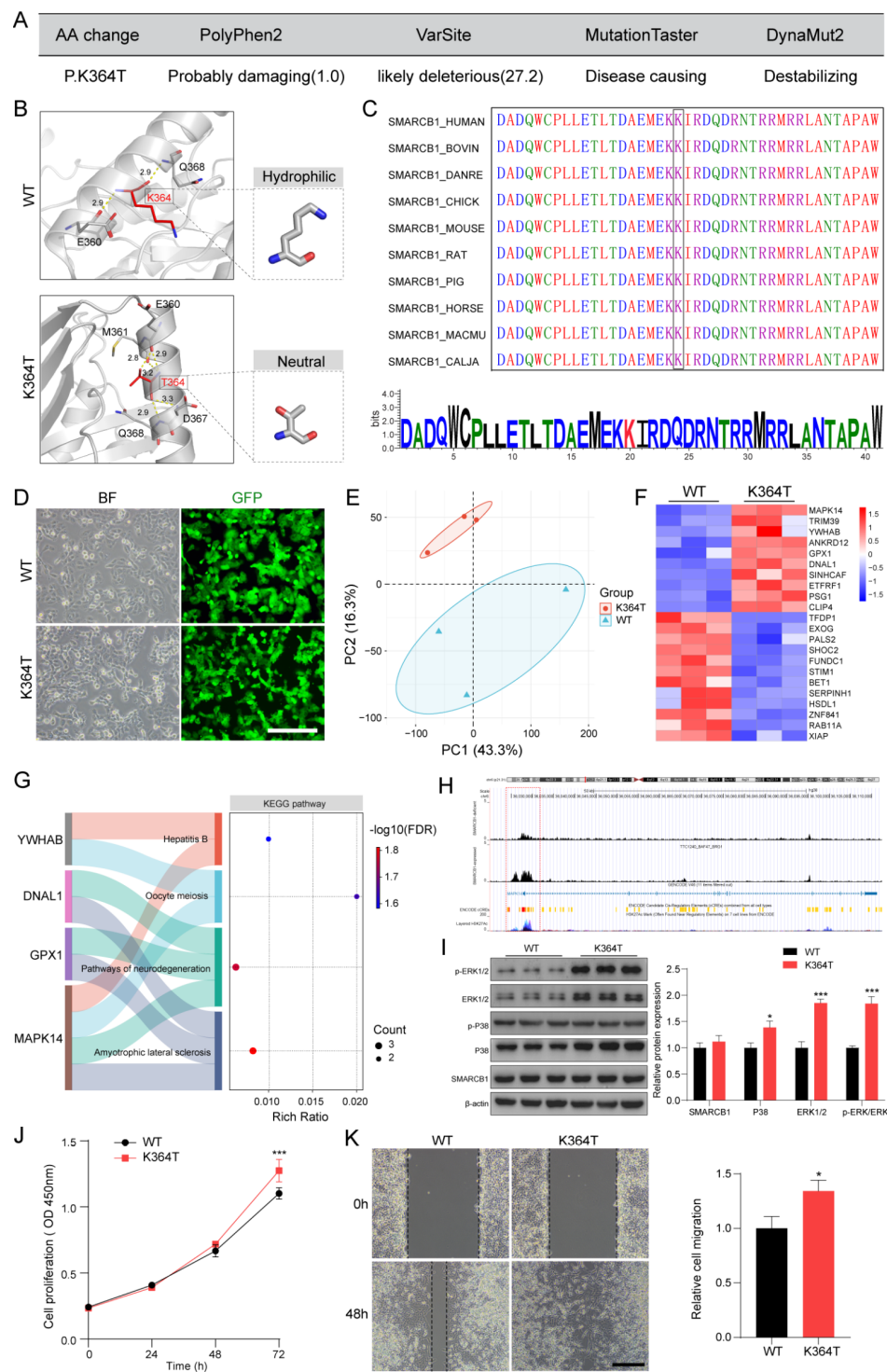


Figure 2. Functional evaluation of the c.1091A>C variant in *SMARCB1* (A) *In silico* predictions of the pathogenicity of P.Lys364Thr (K364T). (B) Three-dimensional *SMARCB1* structure overlayed with wild-type (WT) or K364T substitution. (C) Evolutionary conservation of Lys364 in different mammalian species. (D) Representative bright field and GFP fluorescence images of IOMM-Lee cells infected with lentivirus carrying WT or K364T-mutated *SMARCB1* (scale bar: 100 μ m). (E) Principal component analysis (PCA) of the transcriptome data. (F) Heatmap plot of DEGs in the K364T group versus the WT group. (G) Enrichment of KEGG pathways in significantly upregulated genes. (H) Binding capacity between BRG1 and the promoter of the *MAPK14* gene in the malignant rhabdoid tumor cell line TTC1240, with or without *SMARCB1* expression (CistromeDB: 83223 and 83225). (I) Western blot analysis of the protein expressions of P38-ERK pathway components in the two groups ($n = 3$, $*P < 0.05$, $***P < 0.001$). (J) Growth curves showing the proliferation of WT or K364T-mutant cells ($n = 4$, $***P < 0.001$). (K) Wound healing assay was used to examine the effect of *SMARCB1* mutation on cell migration ($n = 4$, $*P < 0.05$, scale bar: 100 μ m).

to the formation of three additional hydrogen bonds with other amino acids (Figure 3A). In contrast, dolastatin 10 and erdafitinib did not demonstrate significant binding specificity (Figure 3B,C). These findings suggest that RG7112 can bind effectively to the mutant *SMARCB1* protein and may serve as a targeted therapeutic option for patients with *SMARCB1*-mutant tumors or CSS.

Summary of reported *SMARCB1* pathogenic mutations in CSS patients and meningioma patients

Through literature review, we identified 31 patients diagnosed with CSS, including those in our study, who harbored 15 reported germline mutations in *SMARCB1* [7,11–13,37–45] (Figure 4A and Supplementary Table S1). The mutations included 12 missense mutations (80%) and 3 deletion/duplication mutations. The most common variant was c.1091_1093del, which was found in 10 patients, accounting for 32.3% of all patients. This was followed by c.110G>A and c.1121G>A, with 4 and 3 cases, respectively. Moreover, 83.9% of the mutations were classified as *de novo*, although germline mosaicism could not be excluded. In our study, the ages at diagnosis for the patients were 33 and 36 years, whereas those of the other reported patients did not exceed 26 years. Among the documented cases, the male-to-female ratio was 5:12. Notably, most CSS patients exhibit moderate to severe intellectual disability. For example, patients with the c.110G>A variant demonstrate severe intellectual disability and choroid plexus hyperplasia [13]. Con-

versely, a recent study indicated that patients with the c.568C>T mutation exhibited normal intelligence, with only mild selective neuropsychological deficits [37]. Interestingly, 11 of the 15 mutations occurred in the C-terminal domain (CTD) of the *SMARCB1* protein and were specifically concentrated at amino acids 352–377. These amino acids are crucial for the interaction of *SMARCB1* with histones, including H2AC6 and H2BC4, facilitating the chromatin remodeling and gene expression mediated by the SWI/SNF complex (Figure 4B). These findings suggest that mutations in the CTD of the *SMARCB1* protein may impair the function of the SWI/SNF complex, contributing to the pathogenesis of CSS.

Additionally, we identified four germline mutations associated with meningiomas, primarily located in the N-terminal region of the *SMARCB1* protein (Figure 4A and Supplementary Table S1) [46–48]. The average age at diagnosis among the 12 reported patients with meningiomas was approximately 35 years, and meningioma often occurs concurrently with schwannomatosis. To date, reports on the development of tumors in patients with CSS are limited. Notably, we are the first to report that CSS patients carrying the c.1091A>C mutation develop meningiomas simultaneously. Furthermore, among the three patients with the c.1121G>A mutation, one developed schwannomatosis at the age of 26 [39]. These findings suggest that variants in the *SMARCB1* gene may not only cause CSS but also increase the risk of tumor development, potentially influenced by the age at which the patient's phenotype is

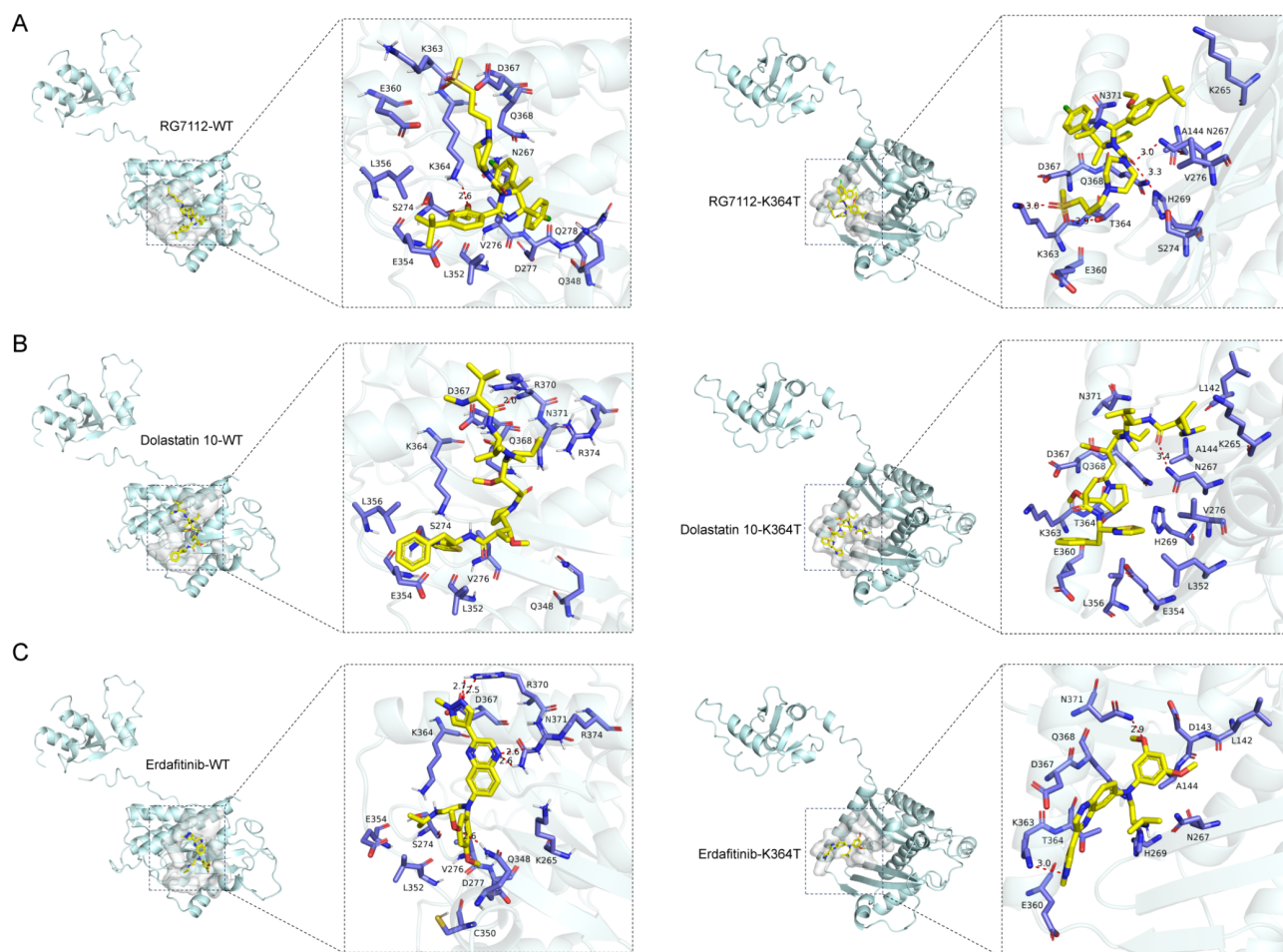


Figure 3. Binding conformations between wild-type/mutated *SMARCB1* and targeted drugs (A) RG7112, (B) dolastatin 10, and (C) erdafitinib.

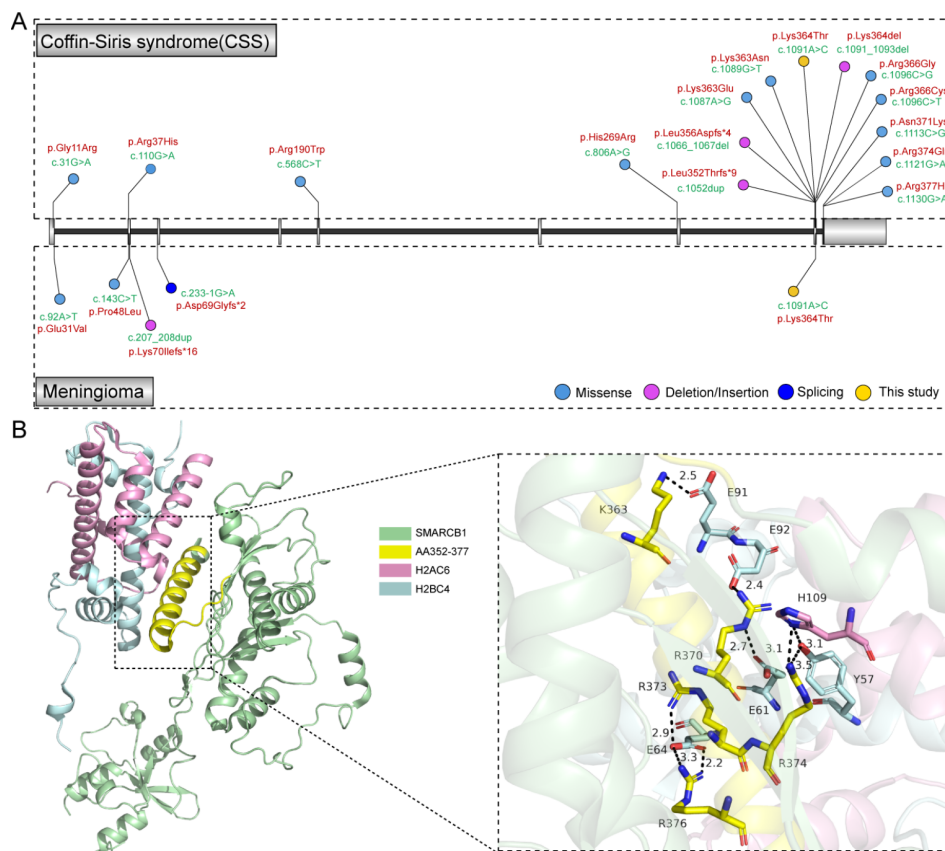


Figure 4. Known pathogenic *SMARCB1* variants in patients diagnosed with CSS3 or meningioma (A) Detailed distribution of reported pathogenic *SMARCB1* variants with base changes in green and amino acid changes in red. (B) Representative images of the crystal structure, including the interactions between *SMARCB1* and H2AC6 or H2BC4.

diagnosed. Currently, there is a lack of phenotypic studies focused on adult CSS patients. Extending the follow-up period may be beneficial for establishing correlations among *SMARCB1* gene mutations, CSS, and tumor development.

Discussion

This study presents a novel missense mutation (c.1091A>C, p. Lys364Thr) in the *SMARCB1* gene, which is associated with Coffin-Siris syndrome type 3 (CSS3) and meningioma in two adult siblings. Our findings broaden the phenotypic spectrum of *SMARCB1*-related disorders and suggest a potential relationship between *SMARCB1* mutations and tumor development.

The pathogenicity of the c.1091A>C mutation was assessed via multiple *in silico* prediction tools, which suggested a likely association with disease. This mutation results in the substitution of lysine with threonine at position 364 (K364T), which alters the protein's hydrophobicity and enhances its interactions within the three-dimensional structure. Lysine 364 is highly conserved across various mammalian species, underscoring its functional importance. Transcriptome analysis of a stable IOMM-Lee cell line expressing the K364T-mutant *SMARCB1* revealed significant alterations in gene expression profiles, notably activating pathways associated with neurodegeneration and tumor progression, such as the P38-ERK pathway. The increased expressions of P38 MAPK, ERK1/2, and phosphorylated ERK1/2, confirmed by western blot analysis, highlights the potential mechanistic link between these

mutations and the pathogenesis of both CSS and meningiomas.

Our study identified the c.1091A>C mutation in two adult patients with CSS3 who exhibited selective difficulties in verbal learning and language delay, in contrast to the severe intellectual disabilities typically reported in CSS3 patients. Additionally, one patient developed meningioma, a finding that has not been previously reported in patients with CSS3. This discovery suggests that *SMARCB1* mutations may predispose individuals to tumor development, potentially influenced by the patient's age at diagnosis and phenotype, which underscores the need for extended follow-up and phenotypic studies in adult CSS patients. Long-term monitoring of patients with *SMARCB1* mutations could provide valuable insights into the progression of CSS and the risk of tumor development, ultimately improving patient management and outcomes.

Given that mutations in the SWI/SNF subunits contribute to the development of cancer and neurodevelopmental disorders, therapeutic manipulation has garnered significant attention. Furthermore, genome-wide perturbation screenings of tumor cells with SWI/SNF mutations have informed drug discovery and clinical trials [2,16,49]. Three strategies for directly targeting SWI/SNF complexes are summarized below: (a) Design small molecules that bind to bromodomains, thereby inhibiting histone post-translational modifications (PTMs) and ultimately blocking the chromatin localization of SWI/SNF complexes, such as BRD9 inhibitors [50,51]. (b) Develop ATPase inhibitors to catalytically inactivate SWI/SNF complexes, such as BRM014 and FHD-286 (NCT04891757)

[52,53]. c) Utilize proteolysis-targeting chimeras (PROTACs) for the targeted degradation of SWI/SNF subunits, including ACB1/2 [54,55], JQ-ds-4 [56], PRT3789 (NCT05639751), CFT-8634 (NCT05355753) and FHD-609 (NCT04965753). Additionally, the activities of polycomb repressive complex 2 (PRC2) [57], DNA damage repair pathways [58], and sensitivity to immune checkpoint inhibition have been identified as therapeutic vulnerabilities [59]. In this study, MOE docking analysis identified RG7112 as a potential therapeutic drug with high binding affinity for the mutated *SMARCB1* protein. This drug targets the MDM2–P53 pathway and is currently undergoing clinical trials for the treatment of leukemia [33]. Moreover, we hypothesize that MAPK pathway inhibitors may also have potential therapeutic effects on *SMARCB1*-mutant meningiomas.

In conclusion, this study highlights the novel association between a *SMARCB1* mutation and CSS3, as well as meningioma, thereby expanding our understanding of *SMARCB1*-related disorders. Further research is essential to elucidate the mechanisms that link *SMARCB1* mutations to tumor development and to explore potential therapeutic interventions for affected individuals.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Ahmad K, Brahma S, Henikoff S. Epigenetic pioneering by SWI/SNF family remodelers. *Mol Cell* 2024, 84: 194–201
- Centore RC, Sandoval GJ, Soares LMM, Kadoch C, Chan HM. Mammalian SWI/SNF chromatin remodeling complexes: emerging mechanisms and therapeutic strategies. *Trends Genet* 2020, 36: 936–950
- Clapier CR, Cairns BR. The biology of chromatin remodeling complexes. *Annu Rev Biochem* 2009, 78: 273–304
- Filatova A, Rey LK, Lechler MB, Schaper J, Hempel M, Posmyk R, Szczaluba K, *et al.* Mutations in *SMARCB1* and in other coffin–siris syndrome genes lead to various brain midline defects. *Nat Commun* 2019, 10: 2966
- Hoyer J, Ekici AB, Ende S, Popp B, Zweier C, Wiesener A, Wohlleber E, *et al.* Haploinsufficiency of *ARID1B*, a member of the SWI/SNF–A chromatin-remodeling complex, is a frequent cause of intellectual disability. *Am J Hum Genet* 2012, 90: 565–572
- Santen GWE, Aten E, Sun Y, Almomani R, Gilissen C, Nielsen M, Kant SG, *et al.* Mutations in SWI/SNF chromatin remodeling complex gene *ARID1B* cause coffin–siris syndrome. *Nat Genet* 2012, 44: 379–380
- Tsurusaki Y, Okamoto N, Ohashi H, Kosho T, Imai Y, Hibi-Ko Y, Kaname T, *et al.* Mutations affecting components of the SWI/SNF complex cause coffin–siris syndrome. *Nat Genet* 2012, 44: 376–378
- Van Houdt J, Nowakowska BA, Sousa SB, van Schaik BDC, Seuntjens E, Avonce N, Sifrim A, *et al.* Heterozygous missense mutations in *SMARCA2* cause nicolaides–baraitser syndrome. *Nat Genet* 2012, 44: 445–449
- Bögershausen N, Wollnik B. Mutational landscapes and phenotypic spectrum of SWI/SNF-Related intellectual disability disorders. *Front Mol Neurosci* 2018, 11: 252
- Kosho T, Miyake N, Carey JC. Coffin–siris syndrome and related disorders involving components of the BAF (mSWI/SNF) complex: historical review and recent advances using next generation sequencing. *Am J Med Genet C Semin Med Genet* 2014, 166: 241–251
- Santen GWE, Aten E, Vulto-van Silfhout AT, Pottinger C, van Bon BWM, van Minderhout IJHM, Snowdowne R, *et al.* Coffin–siris syndrome and the BAF complex: genotype–phenotype study in 63 patients. *Hum Mutat* 2013, 34: 1519–1528
- Tsurusaki Y, Okamoto N, Ohashi H, Mizuno S, Matsumoto N, Makita Y, Fukuda M, *et al.* Coffin–siris syndrome is a SWI/SNF complex disorder. *Clin Genet* 2014, 85: 548–554
- Diets IJ, Prescott T, Champaigne NL, Mancini GMS, Krossnes B, Frič R, Kocsis K, *et al.* A recurrent de novo missense pathogenic variant in *SMARCB1* causes severe intellectual disability and choroid plexus hyperplasia with resultant hydrocephalus. *Genet Med* 2019, 21: 572–579
- Valencia AM, Collings CK, Dao HT, St. Pierre R, Cheng YC, Huang J, Sun ZY, *et al.* Recurrent *SMARCB1* mutations reveal a nucleosome acidic patch interaction site that potentiates mSWI/SNF complex chromatin remodeling. *Cell* 2019, 179: 1342–1356.e23
- Kadoch C, Crabtree GR. Mammalian SWI/SNF chromatin remodeling complexes and cancer: mechanistic insights gained from human genomics. *Sci Adv* 2015, 1: e1500447
- Mittal P, Roberts CWM. The SWI/SNF complex in cancer-biology, biomarkers and therapy. *Nat Rev Clin Oncol* 2020, 17: 435–448
- Wilson BG, Roberts CWM. SWI/SNF nucleosome remodellers and cancer. *Nat Rev Cancer* 2011, 11: 481–492
- Swensen JJ, Keyser J, Coffin CM, Biegel JA, Viskochil DH, Williams MS. Familial occurrence of schwannomas and malignant rhabdoid tumour associated with a duplication in *SMARCB1*. *J Med Genet* 2009, 46: 68–72
- Wang AS, Jamshidi AO, Oh N, Sahyouni R, Nowroozizadeh B, Kim R, Hsu FPK, *et al.* Somatic *SMARCB1* mutation in sporadic multiple meningiomas: case report. *Front Neurol* 2018, 9: 919
- Allen MD, Freund SMV, Zinzalla G, Bycroft M. The SWI/SNF subunit *INI1* contains an N-Terminal winged helix DNA binding domain that is a target for mutations in schwannomatosis. *Structure* 2015, 23: 1344–1349
- Schmitz U, Mueller W, Weber M, Sévenet N, Delattre O, Deimling A. *INI1* mutations in meningiomas at a potential hotspot in exon 9. *Br J Cancer* 2001, 84: 199–201
- Yang W, Wang S, Huo X, Yang K, Guo Z, Li Y, Ji X, *et al.* Novel autosomal recessive SINO syndrome-associated *KIDINS220* variants provide insight into the genotype–phenotype correlation. *Heliyon* 2024, 10: e37355
- Guo Z, Geng M, Huang Y, Han G, Jing R, Lin C, Zhang X, *et al.* Upregulation of wilms’ tumor 1 in epicardial cells increases cardiac fibrosis in dystrophic mice. *Cell Death Differ* 2022, 29: 1928–1940
- Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, *et al.* Accurate structure prediction of biomolecular interactions with alphaFold 3. *Nature* 2024, 630: 493–500
- Guo Z, Jing R, Rao Q, Zhang L, Gao Y, Liu F, Wang X, *et al.* Immortalized common marmoset (*callithrix jacchus*) hepatic progenitor cells possess bipotentiality *in vitro* and *in vivo*. *Cell Discov* 2018, 4: 23
- Zheng LM, Zhang S, Wang XF. The introduction of meningiomas in 2021

- WHO classification of tumors of the central nervous system (5th edition). *Zhonghua Bing Li Xue Za Zhi* 2023, 52(2):107–111
27. Coulthard LR, White DE, Jones DL, McDermott MF, Burchill SA. p38MAPK: stress responses from molecular mechanisms to therapeutics. *Trends Mol Med* 2009, 15: 369–379
 28. Borrie SC, Brems H, Legius E, Bagni C. Cognitive dysfunctions in intellectual disabilities: the contributions of the Ras-MAPK and PI3K-AKT-mTOR pathways. *Annu Rev Genom Hum Genet* 2017, 18: 115–142
 29. Diao W, Qian Q, Sheng G, He A, Yan J, Dahlgren RA, Wang X, *et al*. Triclosan targets miR-144 abnormal expression to induce neurodevelopmental toxicity mediated by activating PKC/MAPK signaling pathway. *J Hazard Mater* 2022, 431: 128560
 30. Johnson MD, Reeder JE, O'Connell M. p38MAPK activation and DUSP10 expression in meningiomas. *J Clin Neurosci* 2016, 30: 110–114
 31. Perreault S, Larouche V, Tabori U, Hawkin C, Lippé S, Ellezam B, Décarie JC, *et al*. A phase 2 study of trametinib for patients with pediatric glioma or plexiform neurofibroma with refractory tumor and activation of the MAPK/ERK pathway: TRAM-01. *BMC Cancer* 2019, 19: 1250
 32. Nakayama RT, Pulice JL, Valencia AM, McBride MJ, McKenzie ZM, Gillespie MA, Ku WL, *et al*. SMARCB1 is required for widespread BAF complex-mediated activation of enhancers and bivalent promoters. *Nat Genet* 2017, 49: 1613–1623
 33. Andreeff M, Kelly KR, Yee K, Assouline S, Strair R, Popplewell L, Bowen D, *et al*. Results of the phase I trial of RG7112, a small-molecule MDM2 antagonist in leukemia. *Clin Canc Res* 2016, 22: 868–876
 34. Verreault M, Schmitt C, Goldwirt L, Pelton K, Haidar S, Levasseur C, Guehennec J, *et al*. Preclinical efficacy of the MDM2 inhibitor RG7112 in MDM2-Amplified and TP53 wild-type glioblastomas. *Clin Cancer Res* 2016, 22: 1185–1196
 35. Krug LM, Miller VA, Kalemkerian GP, Kraut MJ, Ng KK, Heelan RT, Pizzo BA, *et al*. Phase II study of dolastatin-10 in patients with advanced non-small-cell lung cancer. *Ann Oncol* 2000, 11: 227–228
 36. Pant S, Schuler M, Iyer G, Witt O, Doi T, Qin S, Tabernero J, *et al*. Erdafitinib in patients with advanced solid tumours with FGFR alterations (RAGNAR): an international, single-arm, phase 2 study. *Lancet Oncol* 2023, 24: 925–935
 37. Apicella M, Battisti A, Pisaneschi E, Menghini D, Digilio MC, Vicari S. First report of coffin-siris syndrome with SMARCB1 variant, normal intelligence and mild selective neuropsychological deficits: a case report and literature review. *Clin Neuropsychologist* 2024: 1–23
 38. Cheng SSW, Luk H-, Mok MT-, Leung S-, Lo IFM. Genotype and phenotype in 18 Chinese patients withcoffin-siris syndrome. *Am J Med Genet Pt A* 2021, 185: 2250–2261
 39. Gossai N, Biegel JA, Messiaen L, Berry SA, Moertel CL. Report of a patient with a constitutional missense mutation in *SMARCB1*, coffin-siris phenotype, and schwannomatosis. *Am J Med Genet Pt A* 2015, 167: 3186–3191
 40. Han JY, Jang JH, Park J, Lee IG. Targeted next-generation sequencing of korean patients with developmental delay and/or intellectual disability. *Front Pediatr* 2018, 6: 391
 41. Keskinen S, Paakkola T, Mattila M, Hietala M, Koillinen H, Laine J, Haanpää MK. Prenatal coffin-siris syndrome: expanding the phenotypic and genotypic spectrum of the disease. *Pediatr Dev Pathol* 2024, 27: 181–186
 42. Mari F, Marozza A, Mencarelli MA, Lo Rizzo C, Fallerini C, Dosa L, Di Marco C, *et al*. Coffin-siris and nicolaides-baraitser syndromes are a common well recognizable cause of intellectual disability. *Brain Dev* 2015, 37: 527–536
 43. Monroe GR, Frederix GW, Savelberg SMC, de Vries TI, Duran KJ, van der Smagt JJ, Terhal PA, *et al*. Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability. *Genet Med* 2016, 18: 949–956
 44. Sekiguchi F, Tsurusaki Y, Okamoto N, Teik KW, Mizuno S, Suzumura H, Isidor B, *et al*. Genetic abnormalities in a large cohort of coffin-siris syndrome patients. *J Hum Genet* 2019, 64: 1173–1186
 45. Wiczorek D, Bögershausen N, Beleggia F, Steiner-Haldenstätt S, Pohl E, Li Y, Milz E, *et al*. A comprehensive molecular study on coffin-siris and nicolaides-baraitser syndromes identifies a broad molecular and clinical spectrum converging on altered chromatin remodeling. *Hum Mol Genet* 2013, 22: 5121–5135
 46. Bacci C, Sestini R, Provenzano A, Paganini I, Mancini I, Porfirio B, Vivarelli R, *et al*. Schwannomatosis associated with multiple meningiomas due to a familial SMARCB1 mutation. *neurogenetics* 2010, 11: 73–80
 47. Christiaans I, Kenter SB, Brink HC, van Os TAM, Baas F, van den Munkhof P, Kidd AMJ, *et al*. Germline SMARCB1 mutation and somatic NF2 mutations in familial multiple meningiomas. *J Med Genet* 2011, 48: 93–97
 48. Melean G, Velasco A, Hernández-Imaz E, Rodríguez-Álvarez FJ, Martín Y, Valero A, Hernández-Chico C. RNA-based analysis of two SMARCB1 mutations associated with familial schwannomatosis with meningiomas. *neurogenetics* 2012, 13: 267–274
 49. Malone HA, Roberts CWM. Chromatin remodellers as therapeutic targets. *Nat Rev Drug Discov* 2024, 23: 661–681
 50. Clark PGK, Vieira LCC, Tallant C, Fedorov O, Singleton DC, Rogers CM, Monteiro OP, *et al*. LP99: discovery and synthesis of the first selective BRD7/9 bromodomain inhibitor. *Angew Chem Int Ed* 2015, 54: 6217–6221
 51. Theodoulou NH, Bamborough P, Bannister AJ, Becher I, Bit RA, Che KH, Chung C, *et al*. Discovery of I-BRD9, a selective cell active chemical probe for bromodomain containing protein 9 inhibition. *J Med Chem* 2016, 59: 1425–1439
 52. Basurto-Cayuela L, Guerrero-Martínez JA, Gómez-Marín E, Sánchez-Escabias E, Escaño-Maestre M, Ceballos-Chávez M, Reyes JC. SWI/SNF-dependent genes are defined by their chromatin landscape. *Cell Rep* 2024, 43: 113855
 53. Fiskus W, Piel J, Collins M, Hentemann M, Cuglievan B, Mill CP, Birdwell CE, *et al*. BRG1/BRM inhibitor targets AML stem cells and exerts superior preclinical efficacy combined with BET or menin inhibitor. *Blood* 2024, 143: 2059–2072
 54. Farnaby W, Koegl M, Roy MJ, Whitworth C, Diers E, Trainor N, Zollman D, *et al*. BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. *Nat Chem Biol* 2019, 15: 672–680
 55. Kofink C, Trainor N, Mair B, Wöhrle S, Wurm M, Mischerikow N, Roy MJ, *et al*. A selective and orally bioavailable VHL-recruiting PROTAC achieves SMARCA2 degradation in vivo. *Nat Commun* 2022, 13: 5969
 56. Papillon JPN, Nakajima K, Adair CD, Hempel J, Jouk AO, Karki RG, Mathieu S, *et al*. Discovery of orally active inhibitors of brahma homolog (BRM)/SMARCA2 aTPase activity for the treatment of brahma related gene 1 (BRG1)/SMARCA4-Mutant cancers. *J Med Chem* 2018, 61: 10155–10172
 57. Gounder M, Schöffski P, Jones RL, Agulnik M, Cote GM, Villalobos VM, Attia S, *et al*. Tazemetostat in advanced epithelioid sarcoma with loss of INI1/SMARCB1: an international, open-label, phase 2 basket study. *Lancet Oncol* 2020, 21: 1423–1432
 58. Williamson CT, Miller R, Pemberton HN, Jones SE, Campbell J, Konde A, Badham N, *et al*. ATR inhibitors as a synthetic lethal therapy for tumours deficient in ARID1A. *Nat Commun* 2016, 7: 13837
 59. Shen J, Ju Z, Zhao W, Wang L, Peng Y, Ge Z, Nagel ZD, *et al*. ARID1A deficiency promotes mutability and potentiates therapeutic antitumor immunity unleashed by immune checkpoint blockade. *Nat Med* 2018, 24: 556–562