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ORIGINAL ARTICLE

CDGSH Iron Sulfur Domain 2 Deficiency Inhibits Cell Proliferation and Induces Cell Differentiation of Neuroblastoma

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Abstract

CDGSH iron sulfur domain 2 (CISD2) is reported to be highly expressed in several cancers, but the role of it in neuroblastoma has not been identified yet. Here, for the first time, we show that CISD2 is involved in neuroblastoma tumorigenesis and regulates neuroblastoma cell proliferation and differentiation. We found that high CISD2 expression correlated significantly with poor outcome of neuroblastoma patients, as well as advanced neuroblastoma tumor stages. Knockdown of CISD2 greatly repressed neuroblastoma cell proliferation and tumorigenesis both in vitro and in vivo. Further investigation showed that CISD2 deficiency resulted in cell cycle arrest in G1 phase and induced cell differentiation of neuroblastoma. Several Cyclins and Cyclin-Dependent Kinases (CDKs) were down-regulated by CISD2 knockdown, indicating that CISD2 probably regulates cell cycle through those genes. Together, we provide evidence that CISD2 is an indicator for neuroblastoma patients prognosis and is indispensable for neuroblastoma cell proliferation and tumorigenesis; CISD2 deficiency can induce neuroblastoma cell cycle arrest and differentiation. These findings suggest that CISD2 could work as a novel and potential therapeutic target for neuroblastoma treatment.

Keywords CDGSH iron sulfur domain 2 · Neuroblastoma · Cell proliferation · Tumorigenesis · Differentiation

Abbreviations

BrdU	5-Bromo-2-deoxyuridine
CDK	Cyclin-Dependent Kinase
CISD2	CDGSH iron sulfur domain 2
DMEM	Dulbecco's modified Eagle's medium
FBS	fetal bovine serum
shRNA	short hairpin RNA

Introduction

Neuroblastoma is a malignant sympathetic nervous system tumor that usually occurs in kids and 90% of the patients are under 5 years old [1, 2]. It originates from the neural crest cells and is most likely to happen in the adrenal medulla and sympathetic chain [3]. The biological characteristics and clinical feature of neuroblastoma are quite different, they depend on age, location and tissue differentiation extent. Some neuroblastomas may metastasize in the early stage, but some may not and turn to spontaneous regression or transform into benign tumors [4]. The detailed mechanism of neuroblastoma spontaneous regression is still unclear, but previous researches have provided evidence that cell differentiation may involve in this process [5]. Patients with high differentiated neuroblastoma show a better overall survival than patients with low differentiated neuroblastoma [6, 7].

CDGSH iron sulfur domain 2 (CISD2) is a zinc finger protein that mainly locates at the endoplasmic reticulum or mitochondrial membranes. It forms a homo-dimer which harbors two redox-active 2Fe-2S clusters [8, 9]. Deficiency of CISD2 can lead to mitochondrial function dysregulation and subsequently induce neurological genetic disorder Wolfram Syndrome [10, 11]. Recently, CISD2 up-regulation has been found in several cancers, such as breast cancer, hepatocellular carcinoma, laryngeal squamous cell carcinoma and cervical cancer [12–15]. However, little is known about the role of CISD2 in neuroblastoma.

Our study reveals that high CISD2 expression is a poor prognosis indicator in patients with neuroblastoma. Knockdown of CISD2 significantly reduced neuroblastoma

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cell proliferation and tumorigenesis. Further mechanism studies showed that CISD2 silence could induce neuroblastoma cell cycle arrest and differentiation. These findings propose CISD2 as a potential therapeutic target for neuroblastoma treatment.

Results

CISD2 is universally expressed in neuroblastoma cell lines and is a prognostic indicator for neuroblastoma patients. It has been reported that CISD2 expression is elevated in some cancers, but the role of CISD2 in neuroblastoma remains unclear yet. First, we detected CISD2 expression in several neuroblastoma cell lines: BE(2)-C, SK-N-AS, SK-N-DZ, SHEP1 and SH-SY5Y. By western blot and qRT-PCR assays we found that CISD2 is commonly expressed in those cell lines both at protein level and mRNA level (Fig. 1a and b). We also performed database analyses to investigate whether CISD2 is indicative for neuroblastoma progress, using the R2: genomics analysis and visualization platform (https://hgserver1.amc.nl/cgi-bin/r2/main.cgi). The results showed that CISD2 could be a meaningful prognostic indicator for neuroblastoma patients. We analyzed three neuroblastoma databases and drew the progression-free survival Kaplan-Meier curves. From the curves we found that high level of CISD2 showed a strong correlation with poor patient outcome, whereas low level of CISD2 was correlated with good patient overall survival (Fig. 1c). Furthermore, CISD2 expression showed a significant and gradient increase along with the tumor stage advancing from Stage 1 to Stage 4, and is much higher in Stage 4 than in Stage 4S (Fig. 1d). Neuroblastoma tumor in Stage 4S has a relative low malignance, which is more localized with limited dissemination ability and can usually regress spontaneously [16]. The results of Fig. 1d indicate that CISD2 expression is positively correlated with tumor malignance. Retrospective studies have revealed a high survival rate of the under-18-month old age group [17], and consistently we found that CISD2 showed a significant higher expression in children elder than 18 months (Fig. 1e). Furthermore, specimens from dead patients hold remarkable higher CISD2 expression than those specimens from alive patients (Fig. 1f). MYCN is well known as an oncogene in neuroblastoma and is closely associated with the malignance and high risk of neuroblastoma [18]. We checked the relationship between the expression of CISD2 and MYCN, and found that CISD2 expression is positively correlated with that of MYCN (Fig. 1g).

Together these results suggest that CISD2 could be a neuroblastoma prognostic marker and might play an oncogenic role in neuroblastoma development.

CISD2 deficiency inhibits neuroblastoma cell proliferation and tumorigenesis. We then knocked down CISD2 in three neuroblastoma cell lines: BE(2)-C, SK-N-AS and SHEP1, by transducing two shRNA sequences, shCISD2#1 and #2, independently. shGFP was used as the knocking down control. Western blot and qRT-PCR assays were used to confirm the knockdown efficiency. The results showed that the shCISD2#2 knocked down CISD2 expression more successfully, while the shCISD2#1 exhibited a relatively lower efficiency, in BE(2)-C, SK-N-AS and SHEP1 cell lines (Fig. 2a and b). We then studied the cell viability after CISD2 knockdown in the three cell lines using the two shRNA interferences, respectively. Compared with the shGFP groups, shCISD2 groups exhibited significant decrease in cell growth. The shCISD2#2 group showed greater growth inhibition than the shCISD2#1 group, which indicated a dose-dependent influence in cell growth by CISD2 defect (Fig. 2c). For the following experiments, the more effective shCISD2#2 interference was used as a representative CISD2-knockdown method. We also conducted BrdU assays to confirm the effect of CISD2 deficiency in neuroblastoma cells, and got consistent results that the BrdU-positive rates in shCISD2#2 groups were much lower than the corresponding shGFP control groups (Fig. 2d). These indicated that CISD2 is indispensable for neuroblastoma cell growth and cell proliferation.

Furthermore, we examined the in vitro clonogenicity abilities of the BE(2)-C and SK-N-AS cell lines after CISD2 knockdown using soft agar assays. (SHEP1 cells have no clonogenicity and tumorigenesis ability.) The results showed much smaller and less colonies in the shCISD2 groups than in the control groups (Fig. 2e). To provide more relevant in vivo evidence, we performed subcutaneous xenograft assays using NOD/SCID mice. The results showed that knockdown of CISD2 in BE(2)-C cells resulted in much smaller tumors than the control cells. Both the tumor volume and weight were less in the shCISD2 group than the shGFP group (Fig. 2f and g). These results suggested that CISD2 is essential for neuroblastoma tumorigenesis and tumor growth.

CISD2 deficiency induces neuroblastoma cell cycle arrest at G1 phase. To investigate how CISD2 influenced neuroblastoma cell proliferation, we examined the effect of CISD2 on the cell cycle course of neuroblastoma cells. By flow cytometry analysis we found that CISD2 knockdown in BE(2)-C and SK-N-AS cells resulted in G1 phase arrest, as well as remarkable cell population reduce in S phase (Fig. 3a and b). These results indicated that CISD2 is indispensable for neuroblastoma cell cycle progression.

To obtain more insight into how cell cycle progression was affected by CISD2, we further investigated several cell cycle related genes expression which are collectively required for the cell cycle progression from the interphase into the mitotic phase. We carried out qRT-PCR and western blot assays in both BE(2)-C and SK-N-AS cells, and found that CISD2

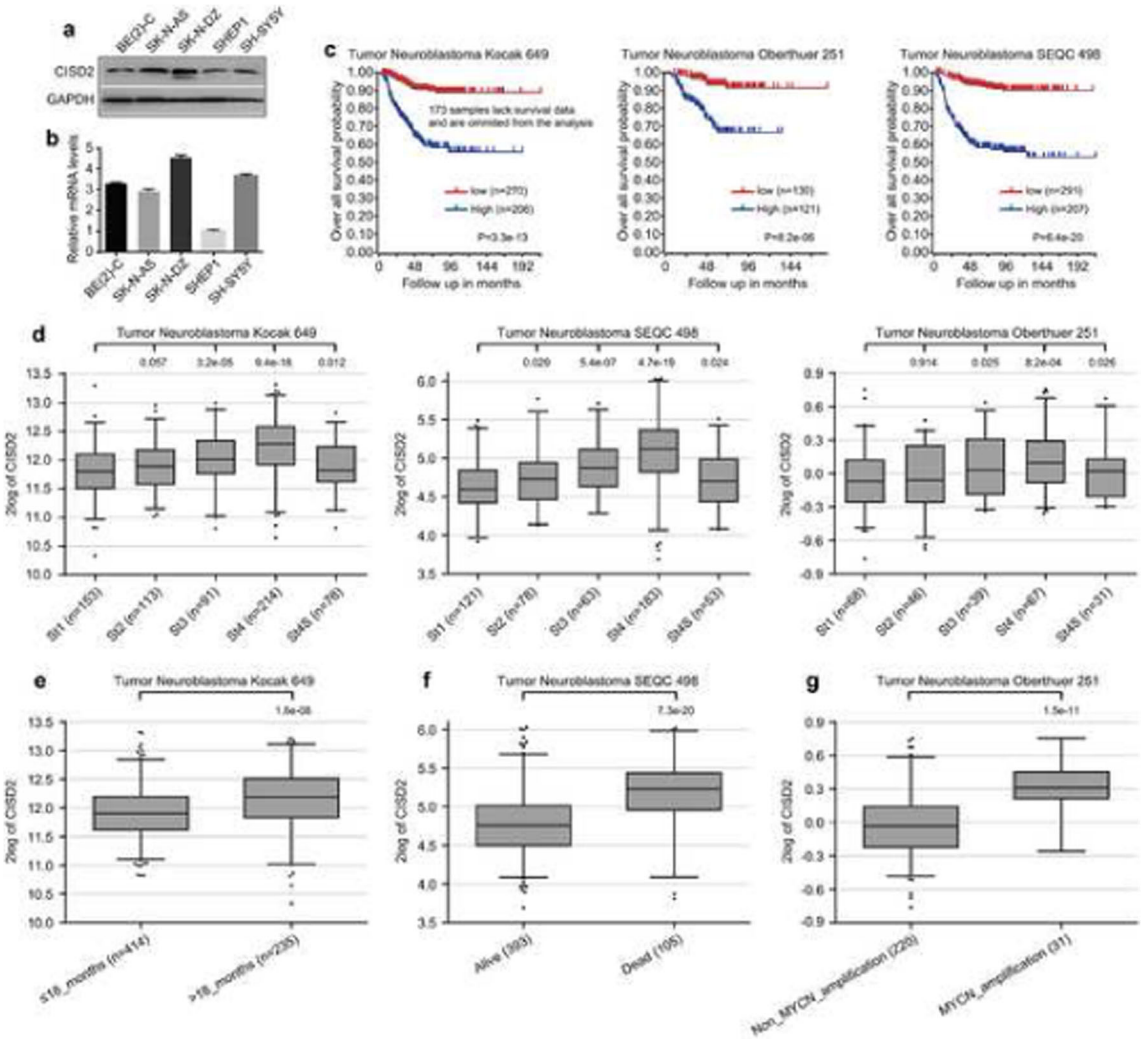

Fig. 1 CISD2 is universally expressed in neuroblastoma cell lines and is a prognostic indicator for neuroblastoma patients **a** Western blot analysis and **b** qRT-PCR analysis of CISD2 expression in five neuroblastoma cell lines. GAPDH was used as loading control. **c** Kaplan-Meier of progression-free survival analysis of three neuroblastoma datasets. **d** Box plot of CISD2 expression levels in Stage (St) 1-4S neuroblastomas. **e** Box plot of CISD2 expression levels in tumors from neuroblastoma patients under or over 18 months. **f** Box plot of CISD2 expression levels in tumors from alive or dead groups. **g** Box plot of CISD2 expression levels in tumors with or without MYCN amplification. The log-rank test *P* values were obtained from the R2 database. 5–95 percentile were shown in the plot in Fig. d, e, f & g

deficiency caused significant decrease in mRNA level of *CDK2, CDK4, CCNB*, *CCND* and *CCNE* (Fig. 3c), as well as notable down-regulation in protein level of CDK2, CDK4, Cyclin B1, CyclinD1 and CyclinE2 (Fig. 3d).

Together, these results provide evidence that CISD2 modulates neuroblastoma cell cycle by regulating the Cyclins and CDKs.

CISD2 deficiency induces neuroblastoma cell differentiation. When culturing the CISD2 silenced cells, we found that in contrast with the control cells, the growth of CISD2 silenced cells was significantly decreased whereas little cells were dead as floating cells. We therefore investigated whether the cell number decrease induced by CISD2 knockdown was related with apoptosis or not. Interestingly, our flow cytometry analysis did not detect cells undergoing apoptosis (Fig. 4a). We further confirmed our results by western blot to examine the apoptosis-associated proteins. We found that neither caspase-3 nor caspase-9 was activated, and the anti-apoptosis protein Bcl-2 did not decrease (Fig. 4b). These results suggested that the cell number decrease was not caused by apoptosis.

Interestingly, after CISD2 was knocked down, BE(2)-C and SK-N-DZ cells displayed observable morphologic features of neuronal differentiation, such as small and rounded cell bodies and extensive outgrowth like neurites and axons (Fig. 4c). The differentiation was further validated by detecting three neuronal differentiation markers: Neurofilament-M, a receptor for glial cell-derived neurotrophic factor, which is critical for radial axon growth and determines axon caliber [19, 20]; Peripherin, which functions in neurite

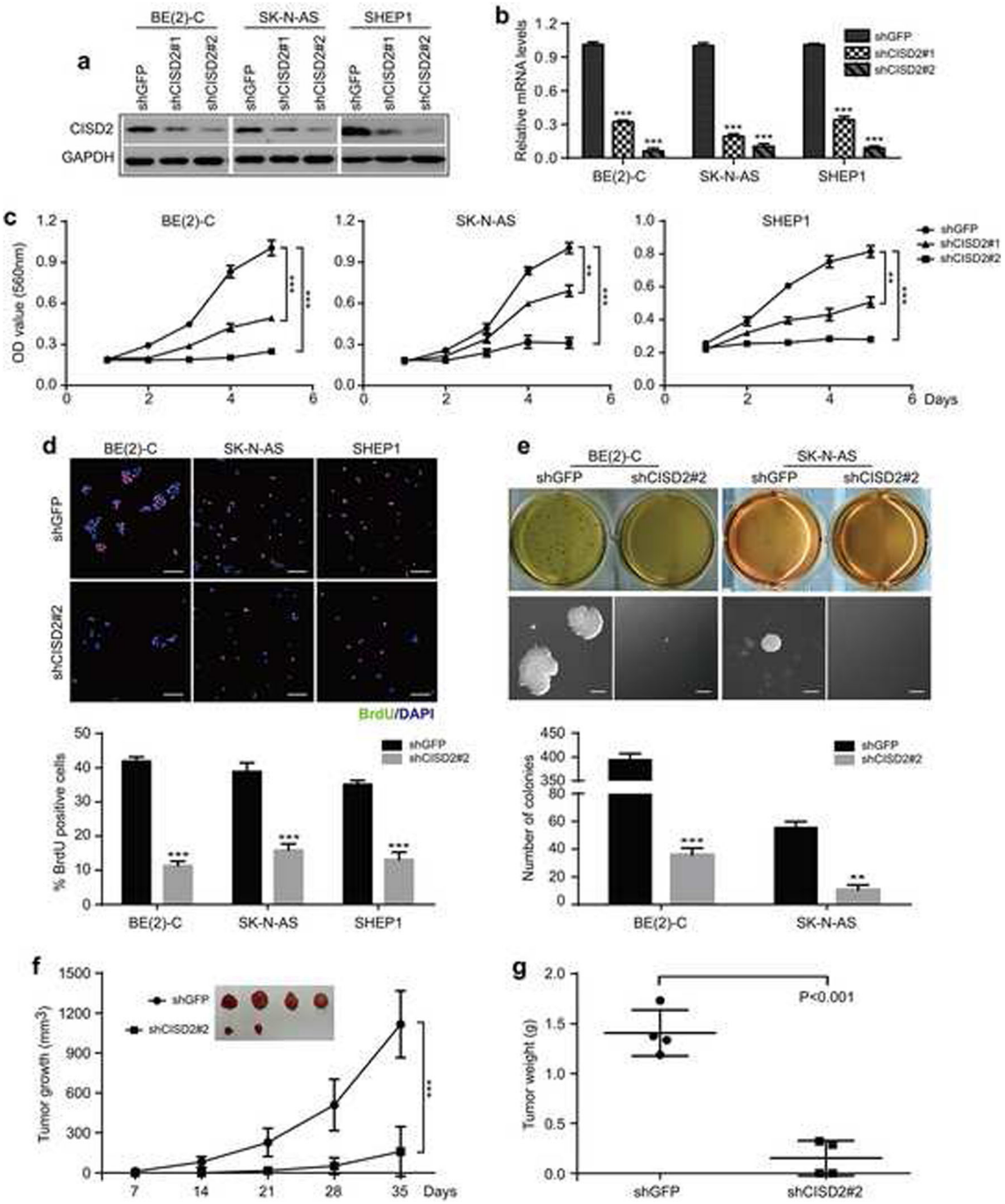

Fig. 2 CISD2 deficiency inhibits neuroblastoma cell proliferation and tumorigenesis **a** Western blot analysis and **b** qRT-PCR analysis of CISD2 expression in BE(2)-C, SK-N-AS and SHEP1 cells expressing shGFP, shCISD2#1 or shCISD2#2. **c** Growth curves of BE(2)-C, SK-N-AS and SHEP1 cells expressing shGFP, shCISD2#1 or shCISD2#2. **d** Representative fluorescence images of BrdU assays and quantification of the indicated BrdU-positive cells. Scale bar = 20 μm. **e** Representative images together with quantification of colony formation of BE(2)-C and SK-N-AS cells with or without CISD2 knockdown. Colony image in the lower panel is a zoom-in from the upper panel. Scale bar = 200 μm. **f** Growth curves together with photographs and **g** weights of the indicated xenograft tumors. GAPDH was applied as loading control. Data were analyzed using two-tailed Student's t-tests. Mean ± SD, $n = 3$ (for Fig. f & g, $n = 4$), ** $P < 0.01$, *** $P < 0.001$

elongation and axonal guidance during development [21] and axonal regeneration [22]; and MASH1, which promotes neuronal differentiation and plays a role in the generation of olfactory, autonomic and retinal neurons [23]. Western blot analysis results showed remarkable increases of these three neuronal differentiation markers, indicating that neuroblastoma cells trended to differentiate after CISD2 was knocked down.

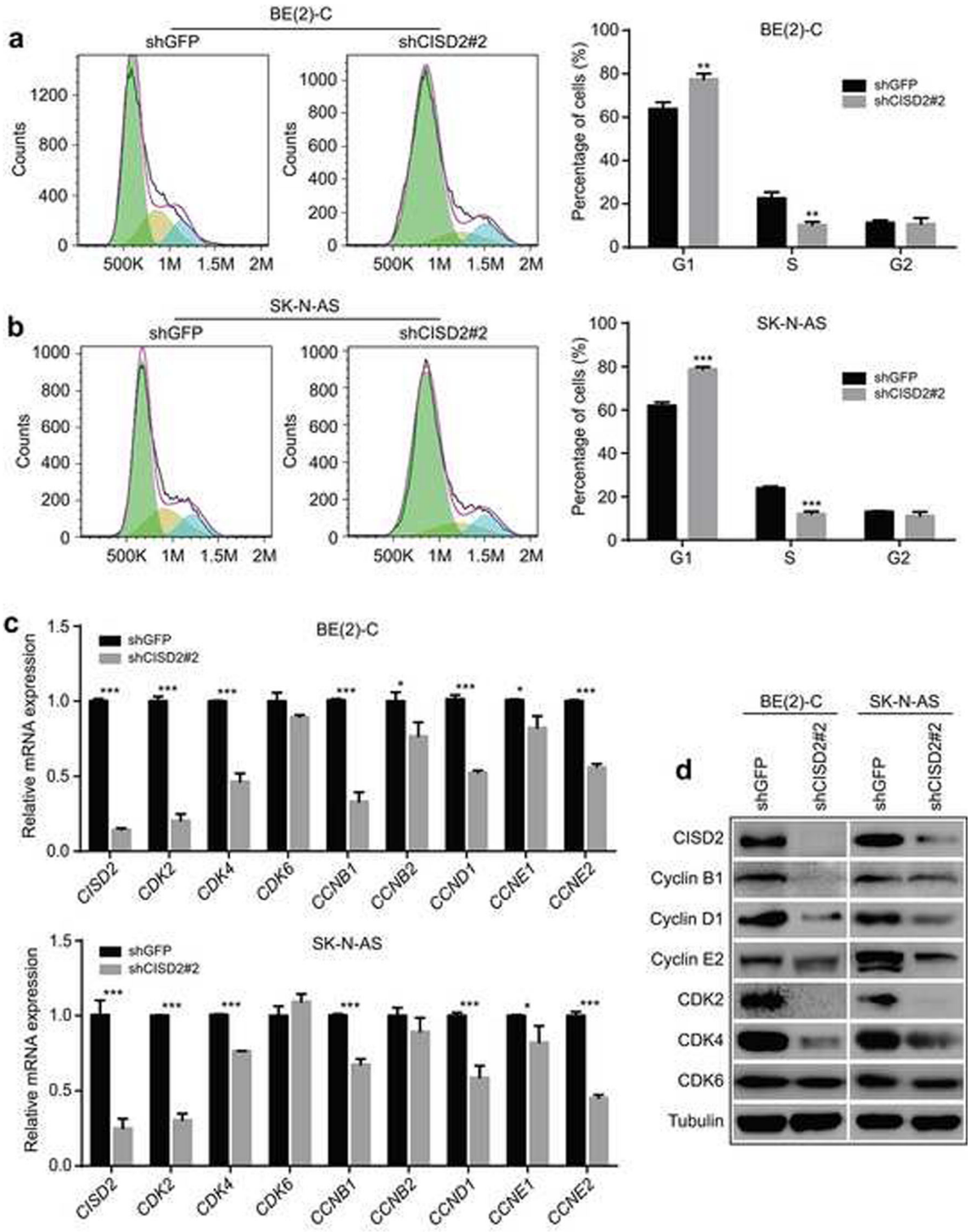

Fig. 3 CISD2 deficiency induces neuroblastoma cell cycle arrest at G1 phase (**a** & **b**) Flow cytometry assays analyzing cell cycle status of **a** BE(2)-C and **b** SK-N-AS cells with or without CISD2 knockdown. Cell population in each phase is quantified and presented. **c** mRNA and **d** protein expression of the indicated genes in BE(2)-C and SK-N-AS cells with or without CISD2 knockdown. Tubulin was used as loading control. Data were analyzed using two-tailed Student's t-tests. Mean ± SD, n = 3, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Discussion

CISD2 is reported as associated with carcinogenesis in many types of human malignant cancers, such as breast cancer, hepatocellular carcinoma, laryngeal squamous cell carcinoma and cervical cancer [12–15]. However, there is no report about the role of CISD2 in neuroblastoma at present. In our study, we for the first time demonstrated that CISD2 is up-regulated

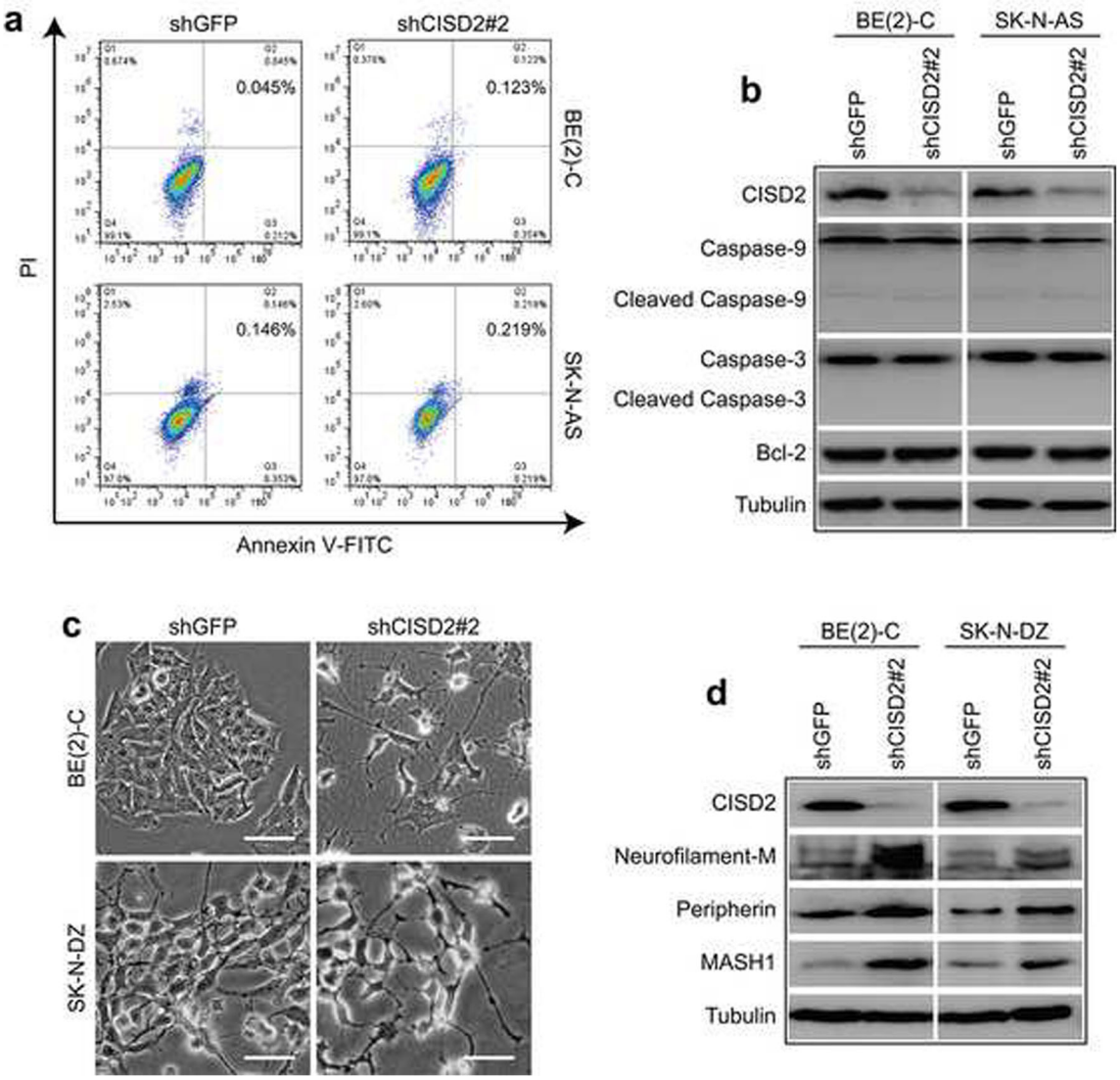

Fig. 4 CISD2 deficiency induces neuroblastoma cell differentiation **a** Flow cytometry assays analyzing apoptosis rate of BE(2)-C and SK-N-AS cells with or without CISD2 knockdown. **b** Western blot analysis of of the indicated proteins expression in BE(2)-C and SK-N-AS cells with or without CISD2 knockdown. **c** Microscopy of the morphology of the BE(2)-C and SK-N-DZ cells with or without CISD2 knockdown. **d** Western blot analysis of of the indicated proteins expression in BE(2)-C and SK-N-DZ cells with or without CISD2 knockdown. Tubulin was applied as loading control. n = 3

in neuroblastoma and is an indicator of poor prognosis. After some functional evaluation we found that knockdown of CISD2 could suppress neuroblastoma cell growth and proliferation, as well as the in vitro colony formation and in vivo tumorigenesis. These suggest that CISD2 is essential for the maintenance of neuroblastoma cell proliferation and tumorigenesis, and consistently in other types of cancers, CISD2 may also play an oncogenic role in neuroblastoma.

CISD2 is reported to regulate various biological processes including cell proliferation, metastasis, autophagy and tumorigenesis [12, 24]. Our study provides evidence for the first time that CISD2 is correlated with neuroblastoma cell proliferation and differentiation. Cyclin E/CDK2 and Cyclin D/CDK4 complexes are required for the cell cycle progression from G1 into S phase [25], and Cyclin B is essential for cell cycle to progress into mitosis phase [26]. Knockdown of CISD2 could reduce these proteins expression and lead to cell cycle arrest, indicating that CISD2 affects cell cycle progression probably through regulating the expression of these Cyclins and CDKs. It is generally known that neuroblastoma in 4S stage has high spontaneous regression rate which indicates favorable prognosis [27, 28], which is likely to correlated with the cell differentiation [5]. Our findings show that the relatively benign Stage 4S neuroblastoma expresses much lower CISD2 than the malignant neuroblastoma in Stage 3–4. Besides, CISD2 is positively correlated with MYCN which is vital for neuroblastoma proliferation and stemness maintenance [29]. Our data show that

down-regulation of CISD2 could induce neuroblastoma cell to transform to a differentiated phenotype and up-regulate the expression of neuronal differentiation markers. These finding suggest that CISD2 may play an important role in neuroblastoma spontaneous differentiation and regression. Together with the cell cycle arrest, we speculate that CISD2 deficiency may block the neuroblastoma cell to proliferate and turn to differentiate.

In conclusion, our findings reveal that CISD2 is essential for sustaining neuroblastoma cell proliferation and tumorigenesis. Down-regulation of CISD2 induces neuroblastoma cell cycle arrest as well as cell differentiation. This study provides supports for CISD2 to serve as a novel and potential molecular target for neuroblastoma therapy.

Materials and Methods

Reagents and Antibodies CISD2 antibody (PAB20855) was from Abnova (USA, CA). Polybrene (sc-134,220) was from Santa Cruz Biotechnology (USA, TX). MASH1 antibody (ab74065), Peripherin antibody (ab4666) and 5′-bromo-2-deoxyuridine (BrdU) antibody (ab6326) were from Abcam (Cambridge, UK). 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) (M 5655), BrdU, and dimethyl sulfoxide (DMSO) (D5879) were from Sigma-Aldrich (St Louis, MO, USA). GAPDH antibody and 4′, 6-diamidino-2-phenylindole (DAPI) were from Beyotime (Haimen, China). The cell cycle antibody sampler kit (#9932), Neuroflament-M antibody (#2838) and cyclin antibody sampler kit (9869) were from Cell Signaling Technologies (Danvers, MA, USA). HRP goat anti-mouse and goat anti-rabbit antibodies were from KPL (Milford, MA, UK). Alexa Fluor 555 Goat Anti-mouse IgG (H + L), Lipofectamine 2000TM, and puromycin (A1113803) were from Life Technologies (IL, USA).

Cell Culture Reagents and medium for cell culture were bought from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). All cell lines used were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Neuroblastoma cell line BE(2)-C was cultured in DMEM/F12 (a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's nutrient mixture F12) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin (P/S). Neuroblastoma cell lines SK-N-AS, SK-N-DZ, SHEP1 and SH-SY5Y, and human embryonic renal cell line 293FT were grown in DMEM supplemented with 10% FBS and 1% P/S. 293FT growth medium was supplemented with extra 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 4 mM L-glutamine and 0.5 mg/ml G418. For transfection experiments P/S and G418 were not supplemented in the 293FT growth medium. All cells were cultured at 37 °C in a 5% CO2 humidified incubator with the duration varying along with different experiments and the medium was replaced every two days.

Western blot and Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Western blot analyses and RT-qPCR assays were performed as previously described [25].

Patient Data Analysis Patient data analyses were performed using the online R2: genomics analysis and visualization platform (http://hgserver1.amc.nl/cgi-bin/r2/main.cgi). Kaplan-Meier survival curves were drawn based on the high vs. low CISD2 expression cutoff determined using the R2 algorithm. *P* values (log-rank test) were obtained from the website. Analysis of patient data was carried out in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study by the database.

Transfection and Infection Lentiviral constructs expressing CISD2 short hairpin (sh) RNA (#1, TRCN0000262370; #2, TRCN0000167018) were purchased from Sigma-Aldrich (St Louis, MO, USA). The pLKO.1-shGFP plasmid as negative control was purchased from Addgene (MA, USA). Lentiviruses were generated as previously described [30].

MTT and BrdU Staining Assays MTT and BrdU staining assays were performed as previously described [30].

Colony Formation Assay Colony formation assays were carried out as previously described [31]. Briefly, 0.6% agarose gel for the lower layer, and 0.3% agarose gel for the upper layer was prepared in a 6-well plate. 1×10^3 cells were mixed with and seeded in the upper gel and were cultured for 3 weeks at 37 °C in a 5% CO2 humidified incubator. 6 fields of the colonies for each group were counted using a microscope.

In Vivo Tumorigenic Assay 4 female non-obese diabetic (NOD)/severe combined immunodeficiency (SCID) mice (5 weeks old; 18~22 g) were applied for the in vivo tumor formation study. Mice raising and experimental procedures and animal welfare were carried out strictly as previously described [31]. Briefly, each mouse was implanted with 5×10^6 shGFP BE(2)-C cells in its left back and 5×10^6 shCISD2 BE(2)-C cells in its right back. Tumor volume was measured to monitor tumor growth (volume = (π/6) x length x width2). The mice were euthanized and the tumors were harvested and weighed after 2 weeks.

Flow Cytometry Analysis Totally 1×10^6 cells were centrifuged at 1000×g at 4 °C for 5 min, and then were washed twice with ice-cold PBS for collection. For cell cycle analysis,

cells were then fixed with 70% ethanol and stained with 20 μg/ml propidium iodide (PI; Thermo Fisher Scientific, Inc.); For apoptosis analysis, cells were then re-suspended in binding buffer containing fluorescein isothiocyanate (FITC)-labeled Annexin V and PI. Following a 25 min incubation at room temperature, the cells were analyzed with a BD FACSVerse flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). BD Cell Quest Pro software version 5.1 was applied for data analysis (BD Biosciences) [32].

Statistical Analysis At least three independent experiments were conducted for biological repeats. Quantitative data were demonstrated as the mean ± standard deviation. Two-tailed and unpaired Student's *t*-tests were performed by GraphPad Prism (version 6.0; GraphPad Software, Inc., La Jolla, CA, USA). $P < 0.05$ indicated a significant difference statistically.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Brodeur GM (2003) Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer 3(3):203–216
2. Takeuchi LA, Hachitanda Y, Woods WG, Tuchman M, Lemieux B, Brisson L, Bernstein M, Brossard J, Leclerc JM, Byrne TD et al (1995) Screening for neuroblastoma in North America. Preliminary results of a pathology review from the Quebec project. Cancer 76(11):2363–2371
3. Anderson DJ (1997) Cellular and molecular biology of neural crest cell lineage determination. Trend Genet: TIG 13(7):276–280
4. Nishi M, Miyake H, Takeda T, Yonemori H, Hanai J, Kikuchi Y, Takasugi N (1994) A trial to discriminate spontaneous regression from non-regression cases during mass screening for neuroblastoma. Jpn J Clin Oncol 24(5):247–251
5. Negroni A, Scarpa S, Romeo A, Ferrari S, Modesti A, Raschella G (1991) Decrease of proliferation rate and induction of differentiation by a Mycn antisense DNA oligomer in a human Neuroblastoma cell-line. Cell Growth Differ 2(10):511–518
6. Manohar CF, Salwen HR, Furtado MR, Cohn SL (1996) Up-regulation of HOXC6, HOXD1, and HOXD8 homeobox gene expression in human neuroblastoma cells following chemical induction of differentiation. Tumour Biol 17(1):34–47
7. Zhang X, Hamada J, Nishimoto A, Takahashi Y, Murai T, Tada M, Moriuchi T (2007) HOXC6 and HOXC11 increase transcription of S100beta gene in BrdU-induced in vitro differentiation of GOTO neuroblastoma cells into Schwannian cells. J Cell Mol Med 11(2): 299–306
8. Conlan AR, Axelrod HL, Cohen AE, Abresch EC, Zuris J, Yee D, Nechushtai R, Jennings PA, Paddock ML (2009) Crystal structure of Miner1: the redox-active 2Fe-2S protein causative in Wolfram syndrome 2. J Mol Biol 392(1):143–153
9. Paddock ML, Wiley SE, Axelrod HL, Cohen AE, Roy M, Abresch EC, Capraro D, Murphy AN, Nechushtai R, Dixon JE, Jennings PA (2007) MitoNEET is a uniquely folded 2Fe 2S outer mitochondrial membrane protein stabilized by pioglitazone. Proc Natl Acad Sci U S A 104(36):14342–14347
10. Chen YF, Kao CH, Kirby R, Tsai TF (2009) Cisd2 mediates mitochondrial integrity and life span in mammals. Autophagy 5(7): 1043–1045
11. Wu CY, Chen YF, Wang CH, Kao CH, Zhuang HW, Chen CC, Chen LK, Kirby R, Wei YH, Tsai SF, Tsai TF (2012) A persistent level of Cisd2 extends healthy lifespan and delays aging in mice. Hum Mol Genet 21(18):3956–3968
12. Liu L, Xia M, Wang J, Zhang W, Zhang Y, He M (2014) CISD2 expression is a novel marker correlating with pelvic lymph node metastasis and prognosis in patients with early-stage cervical cancer. Med Oncol 31(9):183
13. Chen B, Shen S, Wu J, Hua Y, Kuang M, Li S, Peng B (2015) CISD2 associated with proliferation indicates negative prognosis in patients with hepatocellular carcinoma. Int J Clin Exp Pathol 8(10):13725–13738
14. Yang L, Hong S, Wang Y, He Z, Liang S, Chen H, He S, Wu S, Song L, Chen Y (2016) A novel prognostic score model incorporating CDGSH iron sulfur domain2 (CISD2) predicts risk of disease progression in laryngeal squamous cell carcinoma. Oncotarget 7(16):22720–22732
15. Sohn YS, Tamir S, Song L, Michaeli D, Matouk I, Conlan AR, Harir Y, Holt SH, Shulaev V, Paddock ML, Hochberg A, Cabanchick IZ, Onuchic JN, Jennings PA, Nechushtai R, Mittler R (2013) NAF-1 and mitoNEET are central to human breast cancer proliferation by maintaining mitochondrial homeostasis and promoting tumor growth. Proc Natl Acad Sci U S A 110(36):14676–14681
16. Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, De Bernardi B, Evans AE, Favrot M, Hedborg F et al (1994) Revisions of the international criteria for neuroblastoma diagnosis, staging and response to treatment. Prog Clin Biol Res 385:363–369
17. Schmidt ML, Lal A, Seeger RC, Maris JM, Shimada H, O'Leary M, Gerbing RB, Matthay KK (2005) Favorable prognosis for patients 12 to 18 months of age with stage 4 nonamplified MYCN neuroblastoma: a Children's Cancer group study. J Clin Oncol 23(27): 6474–6480
18. Fredlund E, Ringner M, Maris JM, Pahlman S (2008) High Myc pathway activity and low stage of neuronal differentiation associate with poor outcome in neuroblastoma. Proc Natl Acad Sci U S A 105(37):14094–14099
19. Mao L, Ding J, Zha YH, Yang LQ, McCarthy BA, King W, Cui HJ, Ding HF (2011) HOXC9 links cell-cycle exit and neuronal differentiation and is a prognostic marker in Neuroblastoma. Cancer Res 71(12):4314–4324
20. Sihag RK, Jaffe H, Nixon RA, Rong XH (1999) Serine-23 is a major protein kinase a phosphorylation site on the amino-terminal head domain of the middle molecular mass subunit of neurofilament proteins. J Neurochem 72(2):491–499
21. Chang L, Goldman RD (2004) Intermediate filaments mediate cytoskeletal crosstalk. *Nature reviews*. Mol Cell Biol 5(8):601–613
22. Eriksson KS, Zhang SW, Lin L, Lariviere RC, Julien JP, Mignot E (2008) The type III neurofilament peripherin is expressed in the tuberomammillary neurons of the mouse. BMC Neurosci 9

23. Kageyama R, Ishibashi M, Takebayashi K, Tomita K (1997) bHLH transcription factors and mammalian neuronal differentiation. Int J Biochem Cell Biol 29(12):1389–1399
24. Tamir S, Zuris JA, Agranat L, Lipper CH, Conlan AR, Michaeli D, Harir Y, Paddock ML, Mittler R, Cabantchik ZI, Jennings PA, Nechushtai R (2013) Nutrient-deprivation autophagy factor-1 (NAF-1): biochemical properties of a novel cellular target for anti-diabetic drugs. PLoS One 8(5):e61202
25. Huang MY, Xuan F, Liu W, Cui HJ (2017) MINA controls proliferation and tumorigenesis of glioblastoma by epigenetically regulating cyclins and CDKs via H3K9me3 demethylation. Oncogene 36(3):387–396
26. Murray AW (1992) Creative blocks - cell-cycle checkpoints and feedback controls. Nature 359(6396):599–604
27. Evans AE, D'Angio GJ, Randolph J (1971) A proposed staging for children with neuroblastoma. Children's cancer study group a. Cancer 27(2):374–378
28. Haas D, Ablin AR, Miller C, Zoger S, Matthay KK (1988) Complete pathologic maturation and regression of stage IVS neuroblastoma without treatment. Cancer 62(4):818–825
29. Westermark UK, Wilhelm M, Frenzel A, Henriksson MA (2011) The MYCN oncogene and differentiation in neuroblastoma. Semin Cancer Biol 21(4):256–266
30. Xuan F, Huang M, Liu W, Ding H, Yang L, Cui H (2016) Homeobox C9 suppresses Beclin1-mediated autophagy in glioblastoma by directly inhibiting the transcription of death-associated protein kinase 1. Neuro-Oncology 18(6):819–829
31. Yang L, Huang M, Tan J, Hou J, He J, Wang F, Cui H, Yi L (2017) Transcriptional co-activator with PDZ-binding motif overexpression promotes cell proliferation and transcriptional co-activator with PDZ-binding motif deficiency induces cell cycle arrest in neuroblastoma. Oncol Lett 13(6):4295–4301
32. Xuan F, Huang M, Zhao E, Cui H (2018) MINA53 deficiency leads to glioblastoma cell apoptosis via inducing DNA replication stress and diminishing DNA damage response. Cell Death Dis 9(11):1062