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Long non-coding RNA CRNDE promotes tumor growth in medulloblastoma

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Abstract. – OBJECTIVE: Medulloblastoma is the most common malignant brain tumor in children. Despite remarkable advances over the past decades, a novel therapeutic strategy is urgently required to increase long-term survival. This study aimed to understand the role of a long non-coding RNA (lncRNA), colorectal neoplasia differentially expressed (CRNDE), in medulloblastoma tumor growth.

MATERIALS AND METHODS: The transcript level of CRNDE was initially examined in dissected clinical tissues and cultured cancerous cells. Effects of CRNDE knockdown on cell viability and colony formation *in vitro* were assessed using the CCK-8 and colony formation assays, respectively. Cell cycle progression and survival were also determined after CRNDE knockdown. A xenograft mouse model of human medulloblastoma was established by injecting nude mice with medulloblastoma cells stably depleted of CRNDE expression.

RESULTS: Our data suggest that transcript levels of CRNDE are elevated in clinical medulloblastoma tissues instead of in adjacent non-cancerous tissues. Knockdown of CRNDE significantly slowed cell proliferation rates and inhibited colony formation in Daoy and D341 cells. Tumor growth *in vivo* was also inhibited after CRNDE knockdown. Moreover, after knockdown of CRNDE, cell cycle progression was arrested in S phase and apoptosis was promoted by 15-20% in Daoy and D341 cells. *In vivo* data further showed that proliferating cell nuclei antigen (PCNA) was decreased, whereas the apoptosis initiator cleaved-caspase-3 was increased upon CRNDE knockdown in cancerous tissues from the mouse model.

CONCLUSIONS: All these data suggest that CRNDE promotes tumor growth both *in vitro* and *in vivo*. This growth-promotion effect might be achieved via arresting cell cycle progression and inhibiting apoptosis. Therapeutics against CRNDE may be a novel strategy for the treatment of medulloblastoma.

Key Words:
lncRNA, CRNDE, Growth, Medulloblastoma.

Introduction

Medulloblastoma is the most common malignant brain tumor in children. Currently, therapies against medulloblastoma comprise surgery, chemotherapy, and radiotherapy. Due to the remarkable advances over past decades, long-term survival of patients suffering from this malignancy has increased by up to 40-70%[1,2]. However, survivors are often plagued by long-term treatment for neurological complications[3]. Moreover, the incidence rate has been increasingly high over the decades[4]. Thus, novel therapies with more specificity are urgently required.

Colorectal neoplasia differentially expressed (CRNDE) is a novel gene that was initially discovered and identified to be up-regulated in colorectal adenomas and carcinomas[5]. *CRNDE* is located on chromosome 16 on the strand opposite to the adjacent Iroquois homeobox 5 (IRX5) gene, with which it may share a bidirectional promoter. The locus produces transcripts with low coding potential and is therefore classified as a long non-coding RNA (lncRNA)[6]. lncRNAs have recently received sudden attention in research due to increasing reports of their role in human tumorigenesis. Hence, the role of CRNDE in human cancer development and progression has been studied since its discovery. A recent study demonstrated that gVC-In4, one of the CRNDE transcripts that contain a highly-conserved sequence within intron 4, promotes the metabolic changes by which colorectal cancer cells switch to aerobic glycolysis (Warburg effect)[7]. Another study using microarrays[8] has also shown that shRNA-mediated knockdown of CRNDE by targeting exon-containing transcripts (transcript variant 1 and 2)

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led to alteration of various tumorigenesis-associated genes. Although CRNDE was initially identified to be upregulated in colorectal neoplasia, CRNDE expression is now known to be increased in a variety of other cancerous diseases, especially in cancers of the brain[9]. Notably, increased CRNDE expression is typically observed in cancers originating from a cell type or organ[9] where it is not normally expressed, strongly suggesting the critical involvement of CRNDE in human tumorigenesis.

In fact, CRNDE-mediated tumor progression has been revealed recently in glioma. CRNDE is highly expressed in gliomas, particularly in glioblastomas, astroblastomas, and astrocytomas but not in oligodendrogliomas and oligoastrocytomas[10-14]. Consistently, among 129 lncRNAs that were differentially expressed in gliomas, CRNDE was identified as the most highly up-regulated lncRNA (32-fold)[15]. Moreover, CRNDE is highly expressed in primary glioblastomas (3.5-to 6.7-fold up) and glioblastoma cell lines (LN229, LN215, LN319, LN018, and BS149) relative to normal brain cells (16- to 23-fold increased)[16]. Despite different brain tumor types, there tends to be a positive correlation of CRNDE expression with tumor grade and recurrence[9]. *In vitro* data have shown that CRNDE promotes cell growth and invasion in glioma *via* mammalian Target of Rapamycin (mTOR) signaling[17]. More strikingly, CRNDE can also affect the malignant biological characteristics of human glioma stem cells[18]. All these data indicate the potential roles of CRNDE in brain tumors. However, whether CRNDE plays a critical role in medulloblastoma remain largely unknown.

Of note, in addition to tissue-specificity, CRNDE expression also displays a temporal pattern; there is a little expression in most of the adult tissues[9]. This clue might indicate that CRNDE has potential effects on cancers occurring at a younger age. As a malignancy featured to threaten children, we speculate that CRNDE may exert biological activities in tumor growth of medulloblastoma. Herein, we initially detected CRNDE expression in clinical cancerous tissues from patients with medulloblastoma. Furthermore, the effects of CRNDE on tumor growth *in vitro* and *in vivo* were fully investigated using shRNA-mediated knockdown. Thus, our results illuminate the utility of CRNDE as a potential novel therapeutic target for medulloblastoma treatment.

Materials and Methods

Human Samples and Ethical Statement

Fifteen cases admitted to Qingdao Women and Children' Hospital for surgical dissection of medulloblastoma were included. In addition to the tumor, the adjacent non-cancerous tissues were collected for each case. Permission to use the human tissues for research purposes was obtained and approved by an institutional Review Board at the Qingdao Women and Children' Hospital. All patients gave full consent to participate in our study and provided written informed consents. For the animal model, the protocol was approved by the Ethics Committee at Qingdao Women and Children' Hospital, and all efforts were taken to minimize animal suffering.

Cells and Reagents

Medulloblastoma cell lines D283, Daoy, D425, D341, and D458 were obtained from the Shanghai Institute of Biological Sciences, Chinese Academy of Sciences (CAS, Shanghai, China). Cells were maintained in Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Gibco), 100 μg/ml streptomycin, and 100 μg/ml penicillin (Hyclone, Logan, UT, USA). Cells were cultured at 37 °C in a 5% CO_2 humidity-controlled incubator. Primary antibodies against proliferating cell nuclei antigen (PCNA) and cleaved-caspase-3 along with the corresponding secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Specific shRNAs against CRNDE were designed and synthesized by Invitrogen (Carlsbad, CA, USA).

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from cultured cells using Trizol reagent (TaKaRa, Dalian, China) based on the manufacturer's instructions. Thereafter, 2 μg of total RNA was subjected to reverse transcription into cDNA using a PrimeScript RT Master Mix Perfect Real Time (TaKaRa) kit. qRT-PCR was performed using an ABI 7900 real-time RT-PCR system with SYBR Premix Ex Taq Kit (TaKaRa). The transcript levels of CRNDE were normalized to those of GAPDH. The primers for CRNDE and GAPDH were synthesized by GenePharm Co. (Shanghai, China).

Cell Viability Detection

The viability of Daoy and D341 cells with or without CRNDE depletion was assessed using a

cell counting kit-8 (CCK-8) assay (Beyotime, Nantong, China) based on the manufacturer's protocol. Cells were seeded onto 6-cm dishes and transfected with (shRNA group) or without specific shRNA against CRNDE (negative control group, NC group). Subsequently, the cells were trypsinized and resuspended. Cells were then seeded into a 96-well plate at an initial density of 4,000 cells per well. Cells were incubated for another 5 consecutive days. On each day, the cell viability in each well was determined by adding 10 µl of CCK-8 solution. After further incubation at 37 °C for 2 h, absorbance was measured using an ELISA reader at a wavelength of 450 nm.

Colony Formation Assay

Human medulloblastoma cell lines Daoy and D341 were pre-transfected with the specific shRNA against CRNDE and seeded into 12-well plates. Culture medium with 10% FBS was refreshed every two days. After 14 days of culture in 12-well plates, the colonies were stained with crystal violet (0.1%). Cell aggregates of over 50 cells were considered as colonies and the total number of colonies in each group was counted. The colony formation rate was computed using the following formula: colony formation rate = (number of colonies/number of seeded cells) ×100 %.

Cell Cycle Analysis vy Flow Cytometry

Daoy cells and D341 cells were washed with PBS twice and trypsinized, followed by fixation with 70% ethanol for 30 minutes on ice. For degrading RNAs, 20 mg/mL RNase (Sigma-Aldrich, St Louis MO, USA) was added for 1 h at 37 °C. DNA was then stained with 20 mg/ml propidium iodide (PI, Sigma-Aldrich), and was assessed by flow cytometry on a FACS Calibur system (BD Biosciences, San Jose, CA, USA) equipped with the ModiFit LT v2.0 software.

Cell Apoptosis Analysis

Cell apoptosis was analyzed using annexin V/PI apoptosis kit (Invitrogen) according to the manufacturer's instruction. Briefly, Daoy and D341 cells were seeded in a 6-well plate (1×10^6 cells/well) and transfected with the specific shRNA against CRNDE (shRNA group) or with a negative control shRNA (NC group). Both cell lines were then cultured in complete medium for 48h. Thereafter, the cells from each group were washed with ice-cold phosphate buffered saline (PBS), and were resuspended in 100 µl binding buffer at a density of 1×10^7 cells/ml. Then, 5 µl of annexin V-FITC and 5 µl of PI working solution (100 µg/ml) were respectively added to 100 µl aliquots of the cell suspension. Cell suspensions were further incubated at room temperature for 15 min in the dark, and later another 400 µl of binding buffer was added to the cell suspension. Samples were then analyzed by flow cytometry. Each sample was tested thrice in triplicate.

Xenograft Mouse Model of Medulloblastoma

Six-week-old male athymic BALB/c nude mice were maintained in special pathogen-free (SPF). For the model with Daoy cells, 12 mice were divided into two groups, each containing 6 mice. For the model using D341 cells, 7 mice were randomly assigned to each group. Briefly, prior to the experiment, both cell lines were transfected with specific shRNA against CRNDE (shRNA group) or with a negative control shRNA (NC group). The cells (5×10^6) were then injected subcutaneously into the right flank in each group of mice. Tumor dimensions (length, L and width, W) were then measured twice a week for 4 weeks. Tumor volumes (TV) were then calculated as $TV= (L \times W^2)/2$. After 4 weeks, mice were sacrificed and tumor tissues were dissected for subsequent analyses.

Histology and Immunohistochemistry (IHC) Analysis

Tumor tissues from the mouse model were paraffin embedded and then cut into 4 µm sections. Slides were then stained with hematoxylin and eosin (H&E). After H&E staining, slides were subjected to antigen retrieval in a microwave in 0.1 M citric acid solution (pH 6.0) for 10 min, followed by overnight incubation with primary antibodies at 4°C. The corresponding secondary antibody was added and slides were incubated at room temperature for 1 h. The bunding reaction was developed using 0.05% diaminobenzidine (DAB) containing 0.01% H_2O_2. Representative images were taken for each group.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Comparison between groups was performed using the Student's *t*-test. Differences with a two-sided *p*-value of < 0.05 were considered as statistically significant. All results were repeated in at least three independent experiments.

Results

CRNDE is Highly Expressed in Primary Medulloblastoma Tissues and is Differentially Expressed in Cell Lines

Initially, CRNDE transcript levels were assessed in clinical samples. In 15 cases, the qRT-PCR analysis showed that the relative transcript level of CRNDE was approximately 2-fold higher in cancerous tissues than in the adjacent non-cancerous tissues (Figure 1A). In a series of cultured medulloblastoma cell lines, it was observed that CRNDE was differentially expressed in five cell lines, with the highest expression in Daoy cells, followed by D341 cells. CRNDE expression in Daoy cells was almost 24-fold higher than that in D283 cells. This observation allowed the use of Daoy cells and D341 cells as the representative cell lines that were further cultured for subsequent analyses. Collectively, these data suggest that CRNDE is upregulated in primary medulloblastoma samples.

CRNDE was Successfully Depleted by the Synthesized shRNA

To evaluate the role of CRNDE in medulloblastoma cells, a specific shRNA against CRNDE was employed (shRNA group). For control, a negative control shRNA was also synthesized (NC group). It was shown that after transfection with the specific shRNA, Daoy cells exhibited significantly decreased transcript levels of CRNDE, reaching up to 80% knockdown (Figure 2A). Similarly, in D341 cells, transfection with specific shRNA resulted in decreased CRND Etranscript levels to only 30% of the control D341 cells (Figure 2B). These observations indicate the efficiency of our synthesized shRNA.

CRNDE Knockdown Inhibited Cell Viability and Colony Formation in Daoy and D341 Cells

We next assessed whether cell proliferation *in vitro* was affected by CRNDE knockdown. The cell proliferation assay results showed that Daoy cells depleted of CRNDE, exhibited a slower proliferative rate than control Daoy cells. The difference in proliferative rates between groups was significant at 4 days after shRNA transfection (Figure 3A). Likewise, D341 cells were only 50% viable on day 5 after CRNDE knockdown (Figure 3B). Further, colony formation assay was performed which showed that colonies in the shRNA-transected group were visually less in number than those in the NC group in both Daoy and D341 cells (Figure 3C). Counting the colonies further revealed that CRNDE-depleted Daoy cells formed only 37 colonies relative to the 80 colonies formed in control Daoy cells, resulting in a 53% decrease in colony formation. In D341 cells, 48% decrease in colony formation was upon CRNDE depletion (Figure 3D). Moreover, in the 3-dimensional colony formation assay, it was further confirmed that cells with CRNDE depletion were significantly inhibited from forming colonies, with 75% decrease in Daoy cells (Figure 3E) and 53% decrease in D341 cells (Figure

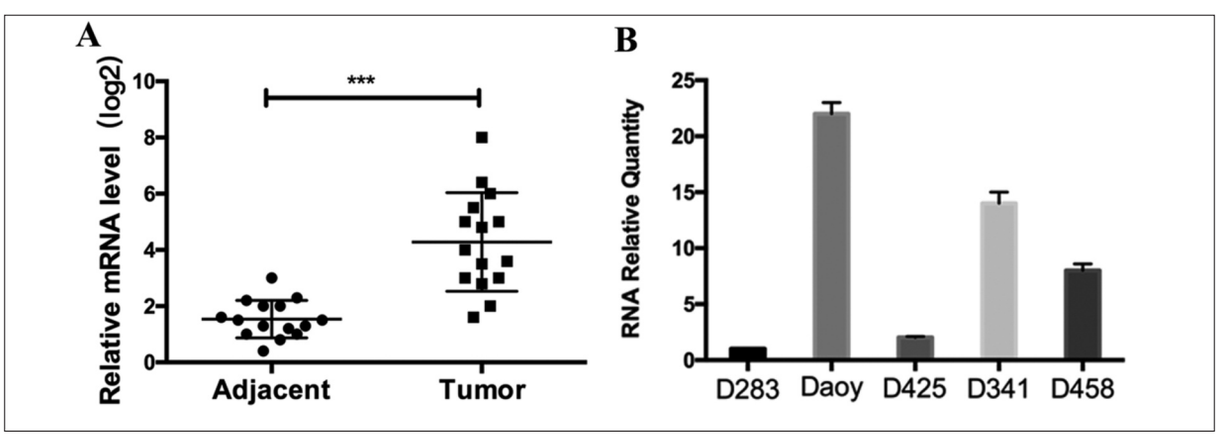

Figure 1. CRNDE was highly expressed in primary medulloblastoma tissues and differentially expressed in cell lines. ***A,*** Fifteen clinical cases diagnosed with medulloblastoma were included and their tumor tissues were collected. Adjacent non-cancerous tissues were also obtained. The transcript level of CRNDE in tumor and adjacent non-cancerous tissues was assessed by qRT-PCR. ***B,*** The relative transcript level of CRNDE in five medulloblastoma cell lines including D283, Daoy, D425, D341, and D458 was assessed by qRT-PCR. ***$p < 0.001$ *vs*. NC group.

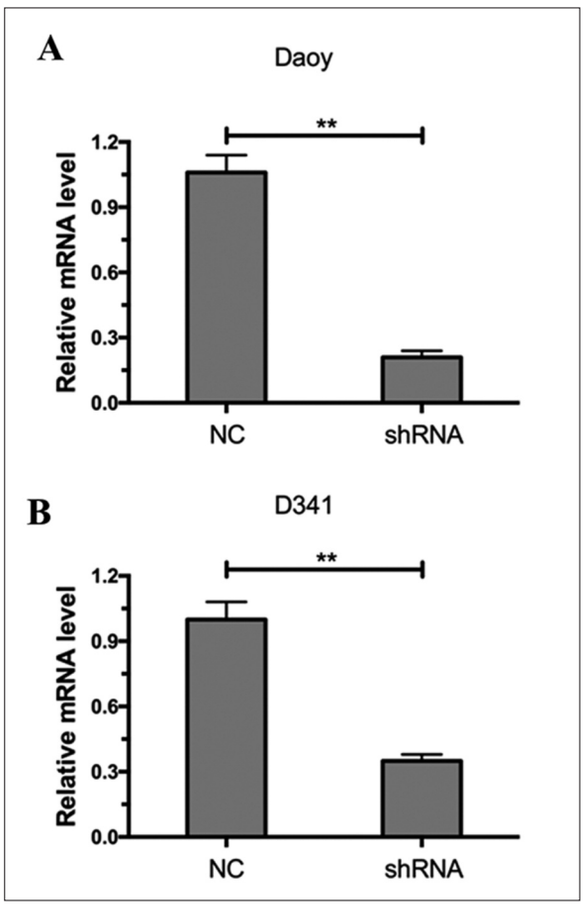

Figure 2. The synthesized shRNA successfully knocked down CRNDE expression in Daoy and D341 cells. ***A,*** After transfection with specific shRNA, Daoy cells exhibited significantly decreased transcript levels of CRNDE, with up to 80% knockdown. ***B,*** Transfection of specific shRNA in D341 cells reduced the transcript level of CRNDE to only 30% of control D341 cells. $^{**}p < 0.001$ *vs*. NC group.

3F). These data strongly suggest that CRNDE knockdown significantly inhibits medulloblastoma cell proliferation *in vitro*.

CRNDE Knockdown Arrested Cell Cycle Progression in S Phase and Induced Cell Apoptosis in Daoy and D341 Cells

We then performed cell cycle analysis with Daoy and D341 cells (Figure 4A). Strikingly, we observed that CRNDE knockdown interrupted cell cycle progression. In Daoy and D341 cells, CRNDE depletion resulted in significant decreases in cell percentages in G0/G1 phase and G2/M phase. Instead, cells were accumulated in the S phase, indicating cell cycle arrest in the S-phase (Figure 4B). Furthermore, apoptosis was evaluated in specific or negative control shRNA-transfected Daoy and D341 cells (Figure 4C). Interestingly, CRNDE-depleted Daoy cells had a significantly higher rate of apoptosis than the control Daoy cells. This was also true in D341 cells where CRNDE-depleted D341 cells showed that 16% of cells were apoptotic, significantly contrasting with the 4% apoptotic rate observed in control D341 cells (Figure 4D). These observations suggest that CRNDE knockdown arrested the cell cycle in S phase and resulted in eventual apoptosis of Daoy and D341 cells.

CRNDE Knockdown Inhibited Tumor Growth and Increased Cleaved-Caspase-3 Expression In Vivo

To evaluate the effect of CRNDE knockdown on tumor growth *in vivo*, a mouse model bearing medulloblastoma was established. In the mouse model bearing Daoy cells, tumor volumes in the CRNDE-depleted group of mice were observed to be significantly smaller than those in control mice starting from week two. The difference in tumor volumes became increasingly remarkable at later time points. In the last week, the tumors were visually smaller in the shRNA group than in the NC group. In fact, average tumor volume in the shRNA group was only 33% of that in the NC group on this specific day (Figure 5A). Similarly, in mice bearing the D341 cells, tumors from the shRNA group were significantly smaller after dissection. Tumor dimensions were significantly decreased in the shRNA group than in the NC group since week three. By week four, the average tumor volume in the shRNA group was only 246 mm^3, which significantly contrasted the 400-mm^3 tumor volume average in the NC group (Figure 5B). These findings suggest that CRNDE knockdown inhibits tumor growth *in vivo*. Furthermore, histological staining with H&E showed that the tumor samples from the NC group were more intensely stained for nuclei with remarkable loss of polarity, whereas tumor samples from the shRNA group were lightly stained for nuclei and displayed regular cell arrangement (Figure 5C). PCNA, a specific indicator of proliferating cells, was mildly positive in the shRNA group, whereas it was strongly developed in the NC group (Figure 5C). All these data verified that CRNDE knockdown inhibited tumor growth *in vivo*. Tumor samples from the mouse model were also processed for the immunofluorescence assay to detect the apoptotic proteins in each group. As shown in Figure 5C, multiple cells exhibited green fluorescence (cleaved-cas-

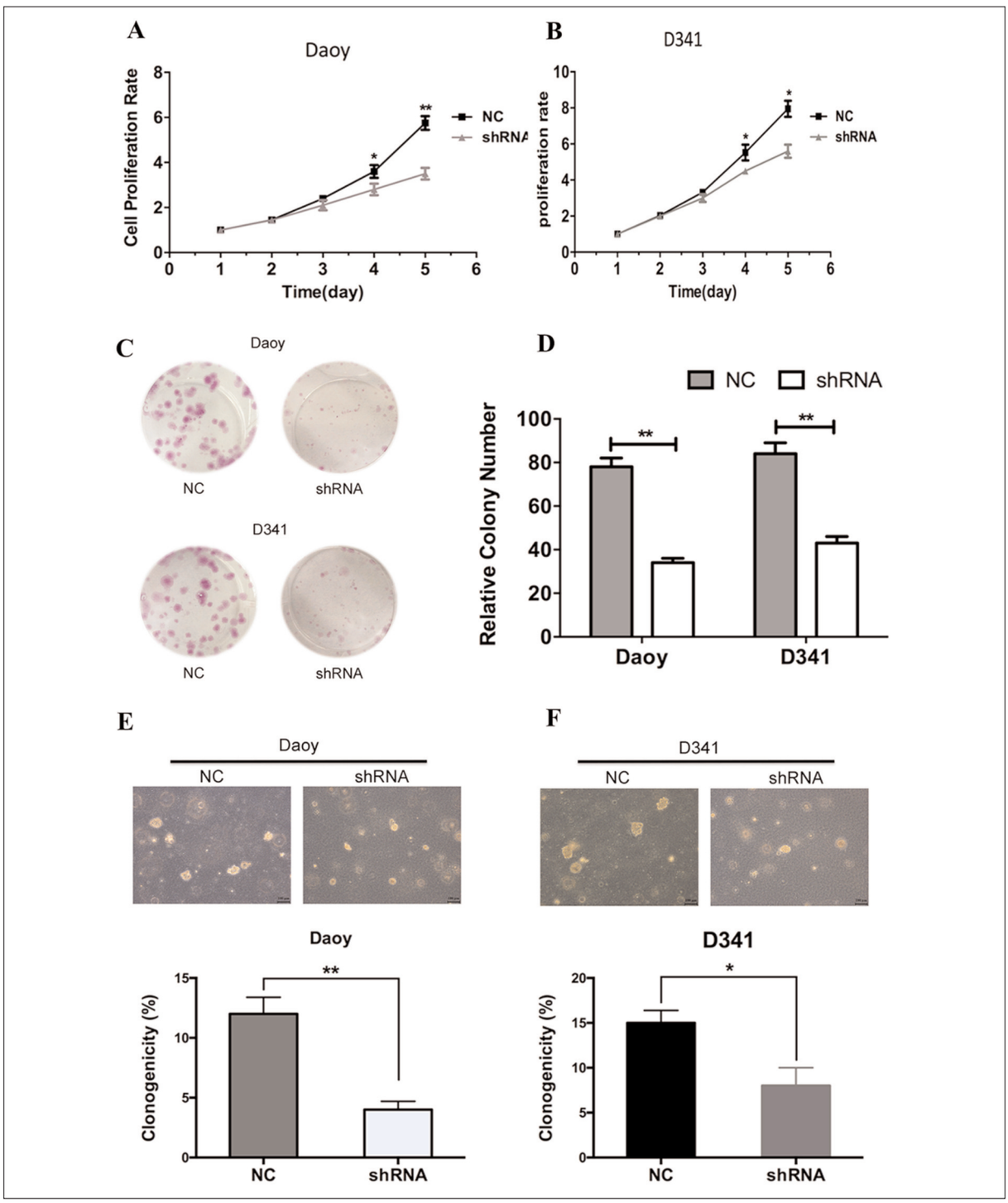

Figure 3. ***A,*** CRNDE knockdown inhibited cell viability and colony formation in Daoy and D341 cells. ***A, B,*** Cell proliferation upon CRNDE depletion (shRNA group) or in controls (negative control group, NC group) was assessed in Daoy and D341 cells. Cell viability was determined over 5 consecutive days. ***C,*** Daoy and D341 cells with or without CRNDE depletion were further subjected to colony formation assay. The colonies in the shRNA group were visually less in number than those in the NC group in both Daoy and D341 cells. ***D,*** Counting the colonies further revealed that CRNDE-depleted Daoy cells formed only 37 colonies relative to 80 colonies in control Daoy cells, resulting in 53% decreased colony formation. A decrease of 48% in colony formation was also observed in D341 cells upon CRNDE depletion. ***E, F,*** In 3-dimensional colony formation assay, it was further confirmed that cells upon CRNDE depletion were significantly inhibited from forming colonies, with a75% decrease in Daoy cells (E) and a 53% decrease in D341 cells. *$p < 0.05$ *vs.* NC group; **$p < 0.01$ *vs.* NC group.

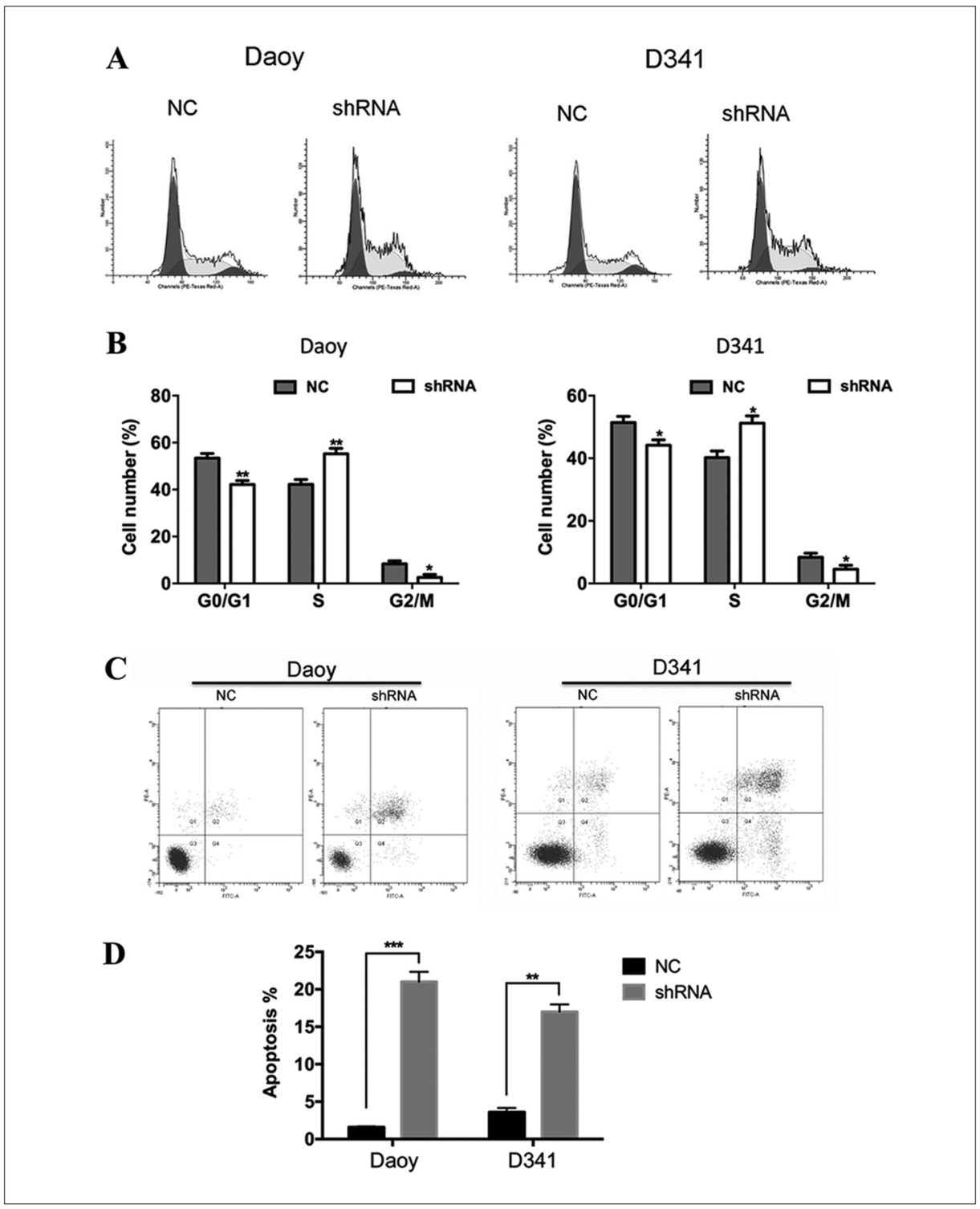

Figure 4. CRNDE knockdown arrested cell cycle in S phase and induced apoptosis in Daoy and D341 cells. ***A,*** Cell cycle progression was analyzed by flow cytometry. ***B,*** In Daoy and D341 cells, when CRNDE was depleted, the cell percentages in G0/G1 phase and G2/M phase were significantly decreased. However, cells were more accumulated in the S phase. ***C,*** Apoptosis was evaluated in specific or negative control (NC) shRNA-transfected Daoy and D341 cells. ***D,*** It is shown that CRNDE-depleted Daoy cells had significantly higher apoptosis rates than control Daoy cells. In D341 cells, 16% of the cells were apoptotic upon CRNDE depletion, significantly contrasting the 4% apoptotic rate in control D341 cells. $^{**}p < 0.01$ *vs*. NC group; $^{***}p < 0.001$ *vs*. NC group.

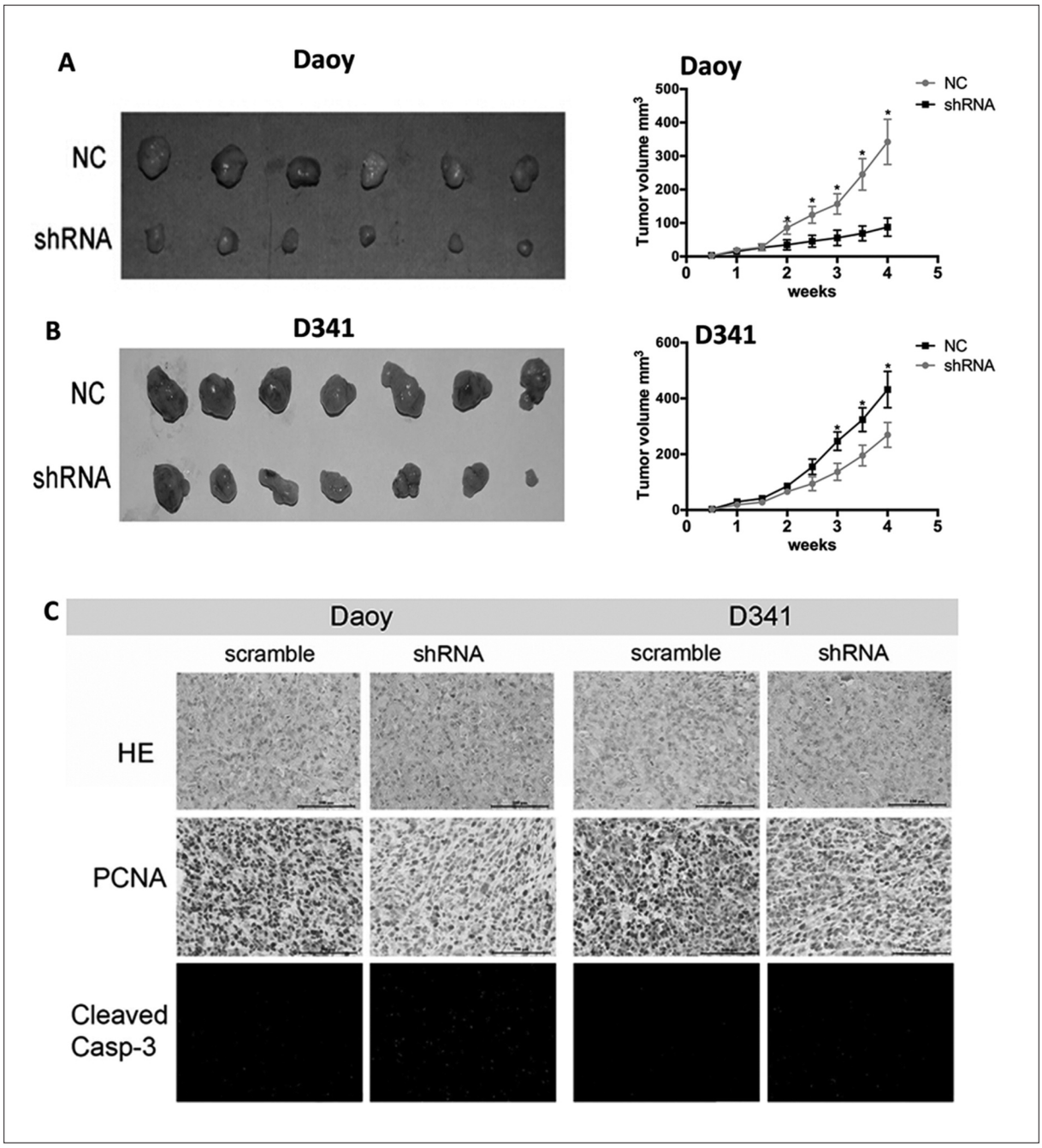

Figure 5. CRNDE knockdown inhibited tumor growth and increased the expression of cleaved-caspase-3 *in vivo*. ***A, B,*** A mouse model bearing medulloblastoma was established using Daoy cells and D341 cells, separately. In the mouse model bearing Daoy cells (n=6 per group), the tumors were visually smaller in the shRNA group than in the NC group after dissection. The average tumor volume in the shRNA group was only 33% of that in the NC group by the end of week 4. Similarly, in mice bearing D341 cells (n=7 per group), tumors from the shRNA group were significantly smaller after dissection. By the end of week four, the average tumor volume in the shRNA group was only 246 mm^3, in contrast to the 400 mm^3 average volume in the NC group. ***C,*** Tumor tissues from each group were further subjected to histological analysis (H&E and immunohistochemistry) and immunofluorescence assay. In H&E staining, tumor samples from the NC group were more intensely stained for nuclei with remarkable loss of polarity, whereas tumor samples from the shRNA group were lightly stained for nuclei and displayed regular cell arrangement. With immunohistochemistry analysis, the proliferating cell nuclei antigen (PCNA), a specific indicator of proliferating cells, was mildly positive in the shRNA group, whereas it was strongly developed in the NC group. In the immunofluorescence assay, multiple cells exhibited green fluorescence (cleaved-caspase-3 positive) in the shRNA group, whereas sporadic cells were fluorescent in the NC group. *$p < 0.05$ *vs*. NC group.

pase-3 positive) in the shRNA group, whereas sporadic cells were fluorescent in the NC group, indicating that depletion of CRNDE promoted the activity of caspase-3, which is an initiator of the apoptotic pathway. This evidence strongly supported our observation that knockdown of CRNDE induced apoptosis *in vitro*.

Discussion

Medulloblastoma is the most common malignant brain tumor during childhood. Although therapy for standard-risk patients has shown improved outcomes, high-risk patients perform poorly[19]. In addition, therapy-related morbidity has been reported, particularly in younger patients[20]. The side effects of therapy form medulloblastoma have, therefore, led to a concentrated search for novel therapeutic targets, of which molecular targeted treatment has elicited great expectations.

The current study investigated the role of the lncRNA CRNDE in medulloblastoma tumor growth. Though initially identified in colorectal cancer, CRNDE was later found to be upregulated in other tumors as well, particularly in brain tumors[9,17]. However, CRNDE expression is tissue-specific and displays temporal patterns. For instance, even within the brain, CRNDE expression varies across different regions, with little expression in most adult tissues[9]. Hence, we speculated that in medulloblastoma, which preferentially occurs in younger patients, CRNDE may be aberrantly expressed. This hypothesis was initially confirmed by detection of relatively increased CRNDE transcript levels in clinically collected tissues. The high expression of CRNDE in medulloblastoma tissues conformed to its upregulation in brain tumors as previously suggested[9]. Furthermore, we investigated whether upregulation of CRNDE confers the oncogenic phenotype. We observed that shRNA-mediated knockdown of CRNDE significantly slowed cell proliferation and inhibited colony formation in Daoy and D341 cells. A mouse model of human medulloblastoma also supported the observation that CRNDE knockdown by shRNA inhabited tumor growth *in vivo*. These *in vitro* and *in vivo* data collectively suggest that CRNDE exerts growth-promotion effects in medulloblastoma. Interestingly, after CRNDE knockdown, the cell cycle was arrested in S phase and apoptosis was eventually induced, indicating that CRNDE promoted tumor growth in medulloblastoma possibly *via* arresting the cell cycle and eventually leading to cell apoptosis.

To date, limited investigations have been performed for the regulatory pathways that contribute to CRNDE function in human diseases. A pioneering study reported that mTOR signaling is critical for the aggressive behavior of CRNDE in glioma[17]. Khalil et al[8] demonstrated a direct interaction between CRNDE transcripts and the components of the polycomb repressive complex 2 (PRC2) and CoREST complex. This interaction has critical roles in epigenetic remodeling of chromatin, indirectly indicating that CRNDE may be involved in epigenetic regulation. In fact, the implication of CRNDE in the epigenetics was also noted in the study by Wang et al[17], where histone acetylation in the promoter region might account for CRNDE upregulation. Notably, a study[21] revealed the positive association between CRNDE levels and epidermal growth factor receptor (EGFR) in high-grade oligodendrogliomas (a type of glioma in which CRNDE is not always up-regulated). The intriguing relationship between CRNDE and EGFR suggested that EGFR pathways might underlie CRNDE functions in human tumorigenesis. Although various pathways are implicated, no pathway is completely probed. Therefore, the participation of CRNDE participation in each specific pathway should be fully investigated in future studies.

Conclusions

We identified CRNDE, a lncRNA, as a critical mediator of tumor growth in medulloblastoma using *in vitro* and *in vivo* assays. The underlying mechanisms contributing to the oncogenic phenotype of CRNDE in medulloblastoma remain to be revealed. We provided evidence that CRNDE might serve as a potential therapeutic target for the treatment of medulloblastoma.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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