Long non coding RNA CCAT2 enhances proliferation, invasion and drug-resistances of non-small lung cancer via activating the miR-204-3p-suppresed IGFBP2/AKT/Bcl2 pathway

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Abstract

Background

The treatment efficacy remains unsatisfactory due to the rapid progress, high rate of metastasis, and drug-resistance of NSCLS. It has been demonstrated that aberrant expression of long non coding RNA colon cancer-associated transcript 2 (CCAT2) in cells was closely related to tumorigenesis, tumor metastasis, and development of drug-resistance. The present study was aimed to thoroughly investigate the effect of CCAT2 in the growth of NSCLC and the underlying mechanisms, so that provide valuable theoretical basis for efficient treatment of NSCLC.

Methods

The expressions of CCAT2 and its target genes and downstream signals were respectively determined by Western-blot assay and RT-qPCR experiment. Cell growth of NSCLC cells was investigated using the CCK-8 kit while the proliferation assay was performed with the help of EDU staining. The NSCLC-bearing mice mode was established to evaluate the effect of PYCR1 on the progress of NSCLC and the underlying mechanisms *in vivo*.

Results

The CCAT2 was highly expressed in NSCLC tumor tissues and cells while not the corresponding normal ones. Further studies revealed that aberrant expression of CCAT2 in lung cancer signally contributed to proliferation, invasion, and migration of cancer cells and the progress of tumor tissues. Moreover, high level of CCAT2 dramatically down-regulated the cytotoxicity of cisplatin (DDP) to NSCLC cells and tissues by upregulation of the drug-resistance related proteins. Mechanisms studies displayed that upregulation of CCAT2 markedly decreased the miR-204-3p while contrary result was obtained when down-regulated the CCAT2 level. We further demonstrated that down-regulation of miR-204-3p level signally enhanced the activity of the insulin-like growth factor (IGF) signaling pathway.

Conclusions

The CCAT2 promoted the progress and drug-resistance of NSCLC thorough activation of the miR-204-3p suppressed IGFBP2/AKT/Bcl2 pathway, and may provide theoretical basis for improvement of therapy of NSCLC.

Background

As one of the most commonly diagnosed cancer types, lung cancer has the highest mortality rates in worldwide [1, 2]. The NSCLC is the most malignant lung cancer type with rapid and progress and

metastasis [3]. Most of NSCLCs were diagnosed at an advanced stage, which paved a rough road for the treatment [4]. The limited effectiveness of current treatments leads to a low 5-year survival rate and an extremely poor prognosis for the NSCLC [5, 6]. Additionally, majority of NSCLC patients inevitably developed the multidrug resistance within a year even they were initially sensitive enough to the treatment of chemotherapy [7–9]. Thoroughly understanding the molecule mechanisms underlying tumorigenesis, progress and drug-resistance is therefore urgently required for enhanced the treatment effect of NSCLC.

Non-coding RNA (ncRNA) represents the most primary human genome, which accounts for nearly 98% of the total amounts [10]. Considerable attention has been provoked on ncRNA since their multiple functions on a broad range of cellular process [11]. Long non-coding RNA (LncRNA), belongs to ncRNAs, represents a class of transcripts with a length of >200 nucleotides [12]. It was previously revealed that abnormal expression of lncRNAs in cells was always correlated to tumorigenesis, tumor progress and tumor metastasis [13]. CCAT2 is the lncRNA that was located at chromosomal 8q24 and composed by 1752 nucleotides [14]. The level of CCAT2 in cells of many tumor types was signally up-regulated, indicated that the CCAT2 was closely associated with risk of several cancers [14]. It was reported that aberrant expression of CCAT2 has been detected in a wide range of cancers, such hepatocellular carcinoma, gastric cancer, and breast cancer [15–17]. CCAT2 plays favorable role in progress and metastasis of many tumor types mainly through promotion of proliferation, invasion, and migration of cancer cells [16].

As the most important endogenous and small noncoding RNAs, micro RNAs (miRNAs) have pivotal role in regulation of gene expression through affect the degradation and/or translational repression of their target messenger (m)RNAs [18, 19]. There are a wide range of miRNAs are involved in the tumorigenesis and development of NSCLC [20]. miR-204, generally composed by two different mature microRNA isoforms miR-204-5p and miR-204-3p, has been demonstrated possess the suppressor role in tumorigenesis [21]. It was widely proved that the miR-204, especially the miR-204-3p, was frequently downregulated in many cancer types and loss of miR-204 expression was favorable for the growth and migration of tumor cells [22]. The miR-204 exhibited diverse functions for inhibition of tumorigenesis through target certain genes and/or signaling pathways [22]. However, the correlation between the miR-204 and development of NSCLC are not thoroughly investigated to date.

In the present study, the levels of CCAT2 in lung cancer tissues and cells were determined and compared with the corresponding normal ones. Besides, the role of CCAT2 in regulation of proliferation, invasion, migration, and drug-resistance of lung cancer were investigated as well. Additionally, the underlying correlation between the miR-204 and NSCLC and its underlying mechanisms were further thoroughly studied.

Materials And Methods

Materials

DDP was obtained from Melonepharma (Dalian, China). The cell counting kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies (Gaithersburg, MD). The RPMI 1640 medium and fetal bovine serum (FBS) were obtained from Gibco (CA, USA) while the 100 U/mL penicillin/streptomycin was purchased from Sigma-Aldrich (St. Louis, MO, USA). The primers were obtained from Sangon Biotech (Shanghai, China) while the primary and/or second anti-bodies were purchased from BD Bioscience (San Diego, CA, USA). All of the other chemicals were analytical or reagent grade.

Cell Lines And Animals

The lung cancer cell lines H520 and A549 and the normal lung tissues cell lines, including BEAS2B, CCD-8L, and LL24 cells, were purchased from the American Type Culture Collection (ATCC). All the cells were cultured in RPMI 1640 containing 10% FBS and supplemented with and 100 U/mL penicillin/streptomycin at 37 °C in a humidified incubator with 5% CO_2. The cisplatin-resistant A549/DDP cell line was obtained from the Bank of Cancer Cell Lines of the Chinese Academy of Medical Science (Beijing, China).

The male Balb/c nude mice, age of 4–5 weeks and weight of 20 ± 2 g, were achieved from BK Lab Anima Ltd. (Shanghai, China). For establishment of tumor-bearing model, 2×10^6 A549 cells were subcutaneously injected into the right flanks of mice. Then the mice were raised under standard condition with free access to food and water. The experimental protocol related animals in the present study was approved by the Animal Experimentation Ethics Committee of Cancer Hospital of China Medical University.

Cell Transfection

For transfection of cells, the plasmids was applied and performed using the Lipofectamine 2000 (Invitrogen, USA) in accordance with the manufacturer's guidelines. After the A549 cells were allowed to grow for 70% confluency in six-well plates, the plasmid plus lipofectamine 2000 was added and co-incubated with cells for 6 h. Then the old medium was removed and immediately replaced with fresh ones and culture for further use.

Colony Formation Assay

The transfected cells were seeded into the six-well plates at the density of 5×10^3 cells/per well. After 10 days of incubation, the cells were stained with 0.1% crystal violet for 20 min. Then the cells were washed three twice with PBS followed by calculation of the numbers of colonies using the Image J software.

Wound-healing Assay

5×10^4 transfected cells were seeded in the six-well plates and allowed to culture for 24 h. Then the old medium was replaced with fresh mediums and the cells were incubated until the full monolayer was formed. Subsequently, a 150 μl sterile polystyrene micropipette tip was applied to make an artificial wound of scratched cells. The scratch were photographed respectively at the 0 h and 48 h using the invert microscope (Chongqing Optical & Electrical Instrument, Co., Ltd., Chongqing, China).

Cell Invasion Assay

For investigation of the vertical migration and invasion ability of cells, the 24-well trans-well (8-μm pore size, Corning, NY, USA) was applied. For migration assay, 5×10^4 cells in 100 μL serum-free medium were seeded in the top chamber while the lower chamber was filled with 600 μL medium containing 10% FBS. After incubation for 24 h, the cells migrated into the lower surface of the insert chamber were stained with 0.1% crystal followed by quantitative analysis using the microplate reader (Thermo Multiskan MK3, USA).

CCK-8 Assay

Cells were seeded in 96-well plates at the density of 5×10^3 cells per well. After 24 h of incubation, the old medium in each well of the plates was replaced with fresh culture medium and continue to incubate for 12, 24, 48 h, respectively, in the presence of DDP or not. Following incubation with 10 μL of CCK-8 reagent for 4 h, the absorbance of each well was detected at 450 nm using the microplate reader (Thermo Multiskan MK3, USA).

Flow Cytometric Analysis

To determine the apoptosis of cells, 5×10^4 transfected A549 cells were seeded in 96-well plates and allowed to grow for overnight. Then the old medium in each well was replaced with fresh serum-free medium containing DDP (0.5 μg/mL). After incubation for 24 h, the cells in each well were harvested by centrifugation at 1000 g for 5 min. Then the apoptosis of cells was examined using the double staining (propidium iodide and annexin V) approach and determined by the FACSscan Flow Cytometer (BD PharMingen, Heidelberg, Germany).

Western Blot Analysis

Total proteins in the tumor tissues and cells was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA) and performed in accordance with the manufacturer's instructions. Then the concentration of RNA was determined using the BCA protein assay kit (Pierce, Thermo Scientific). The tissue or cell lysis solutions were separated by the SDS-PAGE followed by transplant to the polyvinylidene difluoride (PVDF) membranes ((Millipore Corp., Billerica, MA, USA). After that, the membranes were blocked by 5% non-fat

milk in TBST buffer for 1 h followed by co-incubation with primary antibodies (1:1000) for overnight. Subsequently, peroxidase-conjugated secondary antibodies (Yeasen, Shanghai, China) were introduced and co-incubated with the samples for 1 h before visualization of the signal using enhanced chemiluminescence (ECL) method using chemiscope 5600 (CLINX, Shanghai, China). The protein expression levels were normalized to that of GAPDH (Sangon Biotech, Shanghai, China) and semi-quantitative analysis was performed by densitometric scanning.

Real-time Quantitative PCR (RT-qPCR)

The RNA in tissues and cells was obtained as above. After that, the PrimeScript RT Master Mix (Takara, Tokyo, Japan) was applied to conduct the cDNA followed by analysis of the RT-qPCR using the SYBR Green PCR master mix (Takara, Tokyo, Japan). The primer sequences were as follows: CCAT2, forward, GGCCTGTAGGAAGAGTCAAATAG, and reverse, AGGTCAGGAATCAGGAGACA; sh-CCAT2, forward, GATCC GTGCAACTCTGCAATTTAACACTTCCTGTCAGATGTTAAATTGCAGAGTTGCACTTTTTG, and reverse, AATTCAAAAAGTGCAACTCTGCAATTTAACA TCTGACAGGAAGTGTTAAATTGCAGAGTTGCACG; sh-NC, forward, GATCC GCCACTTTGAAGAACCCAATCCTTCCTGTCAGGATTGGGTTCTTCAAAGTGGCTTTTTG, and reverse, AATTCAAAAAGCCACTTTGAAGAACCCAATC TCTGACAGGAAGGATTGGGTTCTTCAAAGTGGCG; AKT forward, GGACAACCGCCATCCAGACT, and reverse, GCCAGGGACACCTCCATCTC; BCl_2 forward, TTCTTTGAGTTCGGTGGGGTC, and reverse, TGCATATTTGTTTGGGGCAGG; IGFBP2F forward, GGGACTGCTTTCCAATAG, and reverse, TTACAGTCTTTGGTCTCGG; GAPDH, forward, CGGAGTCAACG GATTTGGTCGTA, and reverse, AGCCTTCTCCATGGTGGTGAAGAC. The protein expression levels were normalized to that of GAPDH.

Immunofluorescence Assay And Immunohistochemistry (IHC) Study

Tumor tissues were obtained at the end of *in vivo* tumor growth experiments and subjected to preparation of 5 μm slides. Then primary antibodies were introduced and co-incubated with the slides or cells for overnight followed by incubation with streptavidin peroxidase-conjugated or FITC-labeled secondary antibodies. The results were observed by the confocal microscopy analysis (LSM710, Leica, Germany) or invert microscope.

Statistical analysis

The data in the present study were expressed as the means ± SD. The statistical analyses were performed using the GraphPad Prism 7.0 version program. The Student's t-test was applied to evaluate the difference between two means and the comparison between more than two groups was carried out by one-way ANOVA. The statistical significance was defined as $P < 0.05$.

Results

The expression of CCAT2 was up-regulated in NSCLC cells and tissues

We firstly investigated the levels of CCAT2 in NSCLC and also the corresponding normal tissues or cells. As shown in Fig. 1A, obvious higher signal of CCAT2 (brown spots) was detected in the NSCLC tissues compared with the normal adjacent tissues. The levels of CCAT2 expression in NSCLC tissues and the adjacent non-carcinoma tissues were further quantitatively investigated. Results showed that aberrant high expression of CCAT2 was detected in NSCLC tissues while not the non-tumor tissues (Fig. 1B). For cells detection, the results revealed that CCAT2 expression levels were dramatically up-regulated in A549 cells compared with that in normal cells lines, including BEAS2B, CCD-8L, and LL24 cells (Fig. 1C and Fig. 1D). More importantly, it was displayed that the signal of CCAT2 in the drug-resistant cells (A549/DDP) was markedly stronger than that in the non-resistant A549 cells. These results together suggested that CCAT2 might play significant role in the development and inducing drug-resistance of NSCLC.

CCAT2 promoted cell proliferation, migration and invasion of NSCLC cells

The cell transfection efficacy was firstly evaluated by immunofluorescence analysis. As shown in Fig. 2A, obvious stronger green fluorescent intensity was observed in the CCAT2-transfected cells than the control and NC siRNA group. Additionally, the cells treated by si-CCAT2 exhibited the weakest signal of CCAT2 among all groups. Such results were further confirmed by the RT-qPCR assays (Fig. 2B). Colony assay revealed that overexpression of CCAT2 dramatically promoted the proliferation rate of A549 cells, whereas knockdown of CCAT2 expression resulted in obvious decreased the rate (Fig. 2C). Results in Fig. 2D displayed that up-regulation of CCAT2 levels in NSCLC cells contributed to a dramatic increased cell growth rate. However, the growth of A549 cells could be markedly inhibited by transfection with si-CCAT2. The results above together indicated that the CCAT2 has played essential role in the proliferation and growth of NSCLC cells. Subsequently, the migration ability of NSCLC cells was respectively evaluated by wound healing assay and transwell experiment. Results revealed that faster closing of scratch wounds was observed in the cells overexpressed with CCAT2 compared with the CCAT2 negative cells (Fig. 2E). In contrast, the migration speed of cells transfected with si-CCAT2 was dramatically restricted. Moreover, it was also displayed that overexpression of CCAT2 significantly improved the invasion ability of NSCLC cells, with more amount of crystal violet was obtained in the CCTA2 group than others (Fig. 2F).

Over expression of CCAT2 significantly down-regulated the sensitivity of A549 cells to the treatment of DDP

As shown in Fig. 3A, CCK-8 assay revealed that pretreating A549 cells with CCAT2 significantly down-regulated the effect of DDP on inhibition of cell growth. In contrast, the cells treating with si-CCAT2 exhibited obvious higher sensitivity to the toxicity of DDP with a lowest growth rate. The IC50 values for

DDP further confirmed that the aberrant expression of CCAT2 was favorable for inducing drug-resistance in A549 cells, with the group of CCAT2 plus DDP exhibited the highest IC50 values (Fig. 3B). The apoptosis of cells after various treatments was further determined. As shown in Fig. 3C, there was no significant difference between the group of DPP and NC siRNA + DDP. However, the apoptosis rate of cells in the group of CCAT2 + DDP was signally decreased while that in the group of si-CCAT2 + DDP was dramatically elevated. To further confirm the conclusion of CCAT2 inducing drug-resistance of A549 cells, the drug-resistant related proteins, P-glycoprotein (P-gp) and multidrug resistance related protein (MRP), were respectively determined by Western-blot and RT-qPCR assay. As demonstrated in Figure D, after treated by CCAT2 + DDP, the levels of P-gp and MRP in A549 cells was markedly up-regulated. On the contrary, expressions of P-gp and MRP in the cells treated by si-CCAT2 + DDP was the lowest in all groups. Such results were further confirmed by the semi-quantitative analysis and quantitative determination thorough the RT-qPCR experiment (Fig. 3E).

CCAT2 promoted the progress of A549 thorough inhibition of the miR-204-3p

Western-blot analysis revealed that transfection with A549 cells with CCAT2 signally down-regulated the expression of miR-204-3p. In contrast, treating cells with si-CCAT2 dramatically increased the level of miR-204-3p in A549 cells (Fig. 4A). Further quantitative investigation by RT-qPCR confirmed that the level of miR-204-3p in the CCAT2 transfected A549 cells was nearly three times lower than that in the Control and/or NC siRNA group (Fig. 4B). Interestingly, after transfection with the A549 cells with miR-204-3p, the levels of CCAT2 was signally down-regulated (Fig. 4C). Therefore, the correlation analysis was further performed. Results shown in Fig. 4D exhibited that the expression of miR-204-3p was negatively correlated with the CCAT2 expression. These results together suggested that the CCAT2 promoted the progress of A549 thorough inhibition of the miR-204-3p.

Up-regulation Of CCAT2 Activated The miR-204-3p-suppresed IGFBP2/AKT/Bcl2 Pathway

The downstream genes of miR-204-3p was subsequently investigated. As shown in Fig. 5, the cells transfected with CCAT2 exhibited the highest levels of IGFBP2, p-AKT/AKT, and BCl_2 in all groups. In contrast, after transfection of the lung cancer cells with miR-204-3p, the expression of IGFBP2, p-AKT/AKT, and BCl_2 was dramatically decreased. Additionally, there was no significant difference was observed between the group of non-transfected cells and transfected with CCAT2 plus miR-204-3p. These results demonstrated that increase the CCAT2 expression was able of activating the miR-204-3p-suppresed IGFBP2/AKT/Bcl2 pathway.

CCAT2 promoted the growth and drug-resistance of A549 tumor in vivo through silencing the miR-204-3p and activation of the IGFBP2/AKT/Bcl2 pathway

In vivo tumor growth assay (Fig. 6A and 6B) revealed that the mice transplanted with the CCAT2 transfected cells exhibited the fastest tumor growth rate. In contrast, the tumors in the group of si-CCAT2 displayed the slowest growth rate. RT-qPCR analysis of the drug-resistant related proteins showed that the overexpression of CCAT2 signally increased the levels of P-gp and MRP in the tumor tissues (Fig. 6C). Results of IHC analysis displayed that the tumor tissues in the group of CCAT2 have the lowest level of miR-204-3p (Fig. 6D and 6E), which resulted in the highest levels of IGFBP2, p-AKT/AKT, and BCl_2 (Fig. 6F). However, the expression of miR-204-3P in A549 cells could be signally activated by treating with si-CCAT2, and in turn signally down-regulated the activity of IGFBP2/AKT/Bcl_2 pathway.

Further experiments were performed with the presence of DDP. As shown in Fig. 6G and 6H, tumors in the group of CCAT2 + DDP exhibited the fastest deteriorative speed and the highest tumor weight, which similar to the above results. Additionally, results of the survival time revealed that the mice in the group of CCAT2 + DDP achieved the shortest medium survival time while that of the si-CCAT2 + DDP group achieved the longest (Fig. 6I). TNNEL evaluation in Fig. 5J and5K exhibited that the CCAT2 could dramatically down-regulated the cytotoxicity of DDP to A549 tumors. However, down-regulation of the CCAT2 level significantly enhanced the tumor inhibition effect of DDP. Taking these results together, it could be concluded that the CCAT2 enhanced the progress of A549 tumor *via* targeting the miR-204-3p and induced the drug-resistance.

Discussion

Previous studies have demonstrated that lncRNAs cloud be acted as the novel tumor biomarkers so that provide a novel approach for early diagnosis and treatment of a wide range of malignant cancers [23–25]. As an lncRNA that specifically expressed in many cancer types, CCAT2 was proved to be able of accelerated the deterioration of tumors through promotion of the growth and metastasis of tumor tissues and induce chromosomal instability [26]. In the present study, it was revealed that the NSCLC tissues and cells were detected with high level of CCAT2. In contrast, significantly lower signal of CCAT2 was observed in the normal adjacent tissues and various lung cancer tissues. Additionally, the expression of CCAT2 in the cisplatin-resistant lung cancer cells was obvious higher than the non-resistant ones, indicated that CCAT2 might play significant role in the development of drug-resistance for NSCLC.

It was previously revealed that the CCAT2 played significant role in a variety of biological regulation processes, such as cell proliferation, invasion, and migration [14, 15]. Aberrant expression of CCAT2 in tumor tissues and cells was supposed to be the favorable factor that leaded to enhanced growth and metastasis of tumors and proliferation of cancer cells [26]. Our study confirmed that overexpression of CCAT2 dramatically promoted the proliferation and migration rate of lung cancer cells, whereas knockdown of CCAT2 expression resulted in obvious decreased growth rate. Additionally, high level of CCAT2 in NSCLC cells signally contributed to the improvement of invasion ability of NSCLC cells. Moreover, up-regulation of CCAT2 level contributed to the rapidest deteriorate rate of tumor-bearing mice further confirmed the positive role of CCAT2 in the progress of NSCLC

Treatment of lung cancer, especially the NSCLC, always seriously obstructed by the limited strategies, rapid deteriorate rate, and the development of drug-resistance during the treatment late [27]. As the platinum-containing anticancer drug, cisplatin has been recommend for the therapy of multi types of malignant cancer [28]. Although the cisplatin was demonstrated to be one of the most potent chemotherapeutic agent, the development of cisplatin resistance dramatically weaken the treatment efficacy of cancer.29 Multiple mechanisms may resulted the cisplatin resistance, such as excessive drug accumulation, inactivation of agents, and enhanced repair of DNA damage [29, 30]. In our study, we demonstrated that up-regulation of CCAT2 level significantly decreased the inhibition effect of DDP on the growth and inducing apoptosis of NSCLC cells and tissues. On the contrary, knockdown of the CCAT2 expression was favorable for the improvement of tumor inhibition effect of DDP. Molecule detection revealed that increase the level of CCAT2 in NSCLC cells and tissues contributed to significantly elevated expression P-gp and MRP. Such results indicated that the CCAT2 decreased the sensitivity of NSCLC to the treatment of DDP through up-regulation of drug-resistance related proteins.

The miR-204-3p has been found to be deregulated in several tumors, such as hepatocellular carcinoma, glioma, and breast cancer [31, 32], and is closely related with clear cell renal cell carcinoma and upper tract urothelial carcinomas [33]. However, whether there was relationship between the CCAT2 and miR-204-3p remains unclear. In the present study, it was revealed that increase the level of CCAT2 dramatically decreased the miR-204-3p expression in NSCLC cells and tissues. Interestingly, up-regulation of the miR-204-3p level signally down-regulated the activation of CCAT2 in NSCLC cells and tissues. Such negative correlation between the CCAT2 and miR-204-3p suggested that the CCAT2 regulated proliferation, migration, and growth of NSCLC through targeting the miR-204-3p.

The miRNAs regulates multiple cellular process mainly through regulates the expression of its targeted genes and the downstream signaling pathways [18]. There are a wide array of signaling are involved in the tumorigenesis and progress of cancers. Among these, the IGF signaling pathway plays essential role in the embryonic development and also the carcinogenesis [34, 35]. It was found that the IGF-binding protein (IGFBP) superfamily have the inhibitory effects on the activation of IGFs [36]. Of great importance, increasing evidences demonstrated that IGFBP played favorable role in the progress of many carcinogenesis, with aberrant expression of IGFBP and its downstream genes, including p-AKT/AKT and BCl2, signally promoted cancer cell survival and migration [32]. Whether the miR-204-3p inhibited the growth of NSCLC through targeting silence the bioactivity of IGFBP2/AKT/Bcl2 signaling is not clear. In our study, it was revealed that down-regulation of miR-204-3p by transfection with CCAT2 signally activated the levels of IGFBP2, p-AKT/AKT, and BCl2 in lung cancer cells and/or tissues. In contrast, up-regulation of miR-204-3p resulted in dramatically decreased bioactivity of IGFBP2/AKT/Bcl2 pathway and in turn contributed to a relative ideal inhibition effect on tumor growth.Taking together, the present study demonstrated that CCAT2 promoted progress of non-small lung cancer via activating the miR-204-3p-suppresed IGFBP2/AKT/Bcl2 pathway.

In conclusion, the present study demonstrated that the CCAT2 was overexpressed in lung cancer tissues and cells while not the normal ones. Additionally, the aberrant expression of CCAT2 closely related to the

proliferation, invasion, and migration of in NSCLC cells and rapid progress of NSCLC tissues. Moreover, elevation of the CCAT2 level in NSCLC significantly down-regulated the ability of DDP to inhibit the growth of NSCLC cells and tissues. In contrast, decreased the CCAT2 expression dramatically contributed to the improvement of chemotherapy effect of DDP. Negative correlation between the expression of CCAT2 and miR-204-3p indicated the miR-204-3p was the target gene of CCAT2. Taken together, we demonstrated that the CCAT2 promoted the growth of NSCLC thorough targeting the miR-204-3p and induced drug-resistance for DDP.

Conclusion

In conclusion, the present study demonstrated that the CCAT2 was overexpressed in lung cancer tissues and cells while not the normal ones. Additionally, the aberrant expression of CCAT2 closely related to the proliferation, invasion, and migration of in NSCLC cells and rapid progress of NSCLC tissues. Moreover, elevation of the CCAT2 level in NSCLC significantly down-regulated the ability of DDP to inhibit the growth of NSCLC cells and tissues. In contrast, decreased the CCAT2 expression dramatically contributed to the improvement of chemotherapy effect of DDP. Negative correlation between the expression of CCAT2 and miR-204-3p indicated the miR-204-3p was the target gene of CCAT2. Taken together, we demonstrated that the CCAT2 promoted the growth of NSCLC thorough targeting the miR-204-3p and induced drug-resistance for DDP.

Abbreviations

NSCLC, non-small cell lung cancer; CCAT2, colon cancer-associated transcript 2; IGFBP, IGF-binding protein; DDP, cisplatin; ncRNA, non-coding RNA; LncRNA, long non-coding RNA; CCK-8, cell counting kit-8; RT-qPCR, real-Time Quantitative PCR; IHC, immunohistochemistry; P-gp, p-glycoprotein; MRP, multidrug resistance related protein; IGF, insulin-like growth factor.

Declarations

Authors' contributions

Zheng Wang was responsible for the guarantor of integrity of the entire study and preparation, editing, and review of the manuscript. Rongjie Yang and Yu Liu were respectively involved in the definition of intellectual content and literature research. Yangfeng Hang responsible for the study design, data acquisition and analysis.

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Availability of data and materials

The data are available from the authors upon request.

Ethics approval and consent to participate

The experimental protocol related animals in the present study was approved by the Animal Experimentation Ethics Committee of Cancer Hospital of China Medical University.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures
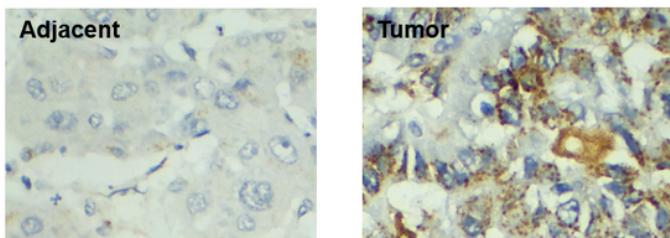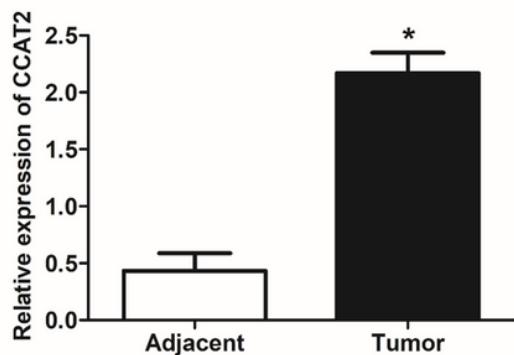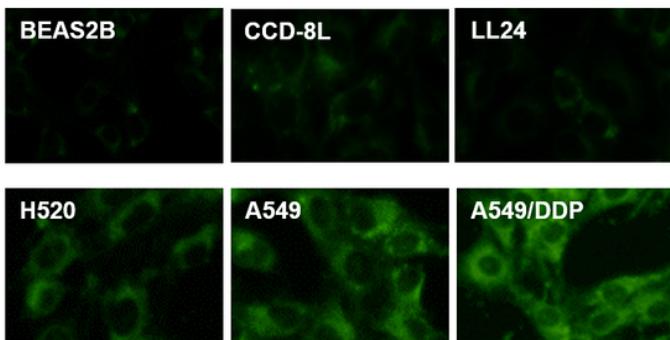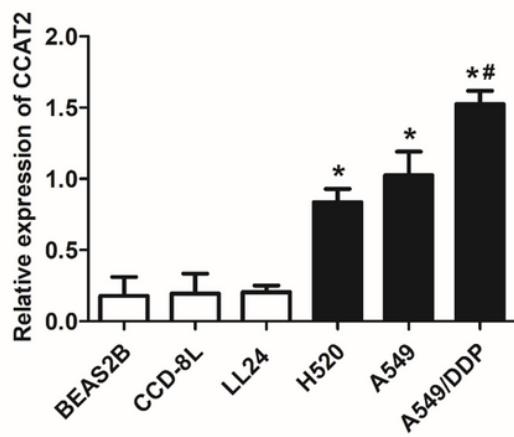
Figure 1

Aberrant overexpression of CCAT2 was observed in non-small lung cancer tissues and cells. (A) Qualitative analysis of the CCAT2 expression in tumor tissues using the immumohistochemical staining approach and compared with the adjacent normal tissues. (B) Determination of the CCAT2 expression in lung cancer tissues and non-tumor tissues by RT-qPCR assay. (C) Immunofluorescence analysis of the CCAT2 expression in lung cancer cell lines H520, A549, and A549/DDP and compared with that in the normal lung tissues cell lines, including BEAS2B, CCD-8L, and LL24 cells. (D) Relative mRNA expression levels of CCAT2 in various lung cancer cells and various normal cells from normal lung tissues determined by RT-qPCR assay. *P<0.05, significantly higher than the normal cells or adjacent non-tumor tissues. #P<0.05, significantly lower than the non-resistant A549 cells.
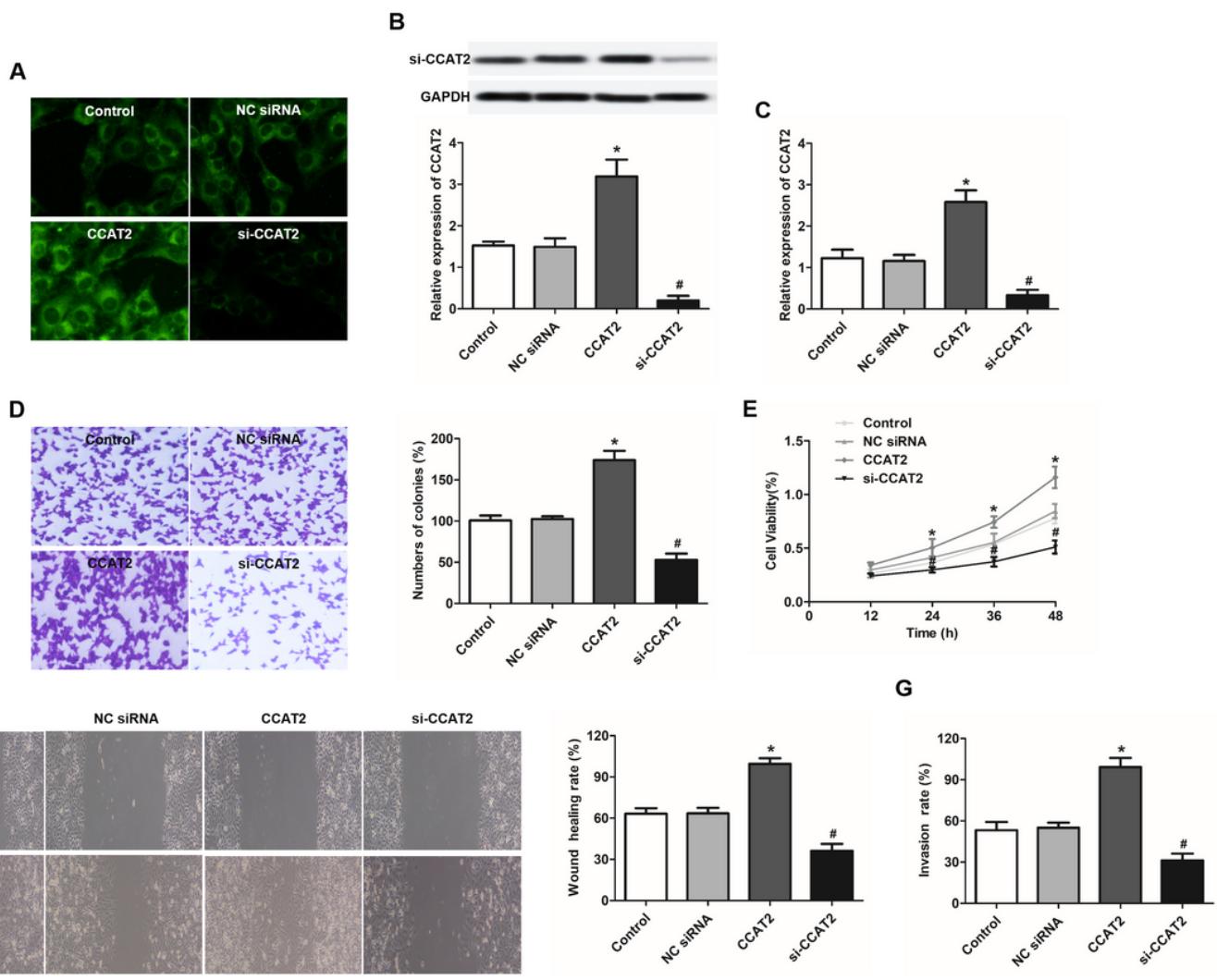
Figure 2

CCAT2 promoted cell proliferation, migration and invasion of NSCLC cells. The transfection efficacy was evaluated by immunofluorescence analysis (A), Western-blot experiment (B), and RT-qPCR assay (C). (D) Colony formation experiments after the A549 cells treated by various strategies. (E) Cell viability of A549 cells at different time points post treatments of NC siRNA, CCAT2, and si-CCAT2, respectively. (F) The migration ability of A549 cells post different treatments was evaluated by wound healing assay. (G) Investigation of the invasion ability of A549 cells post different treatments by trans-well assay. *P<0.05, significantly higher than the control group or cells treated with NC siRNA. #P<0.05, significantly lower than the control group or cells treated with NC siRNA.
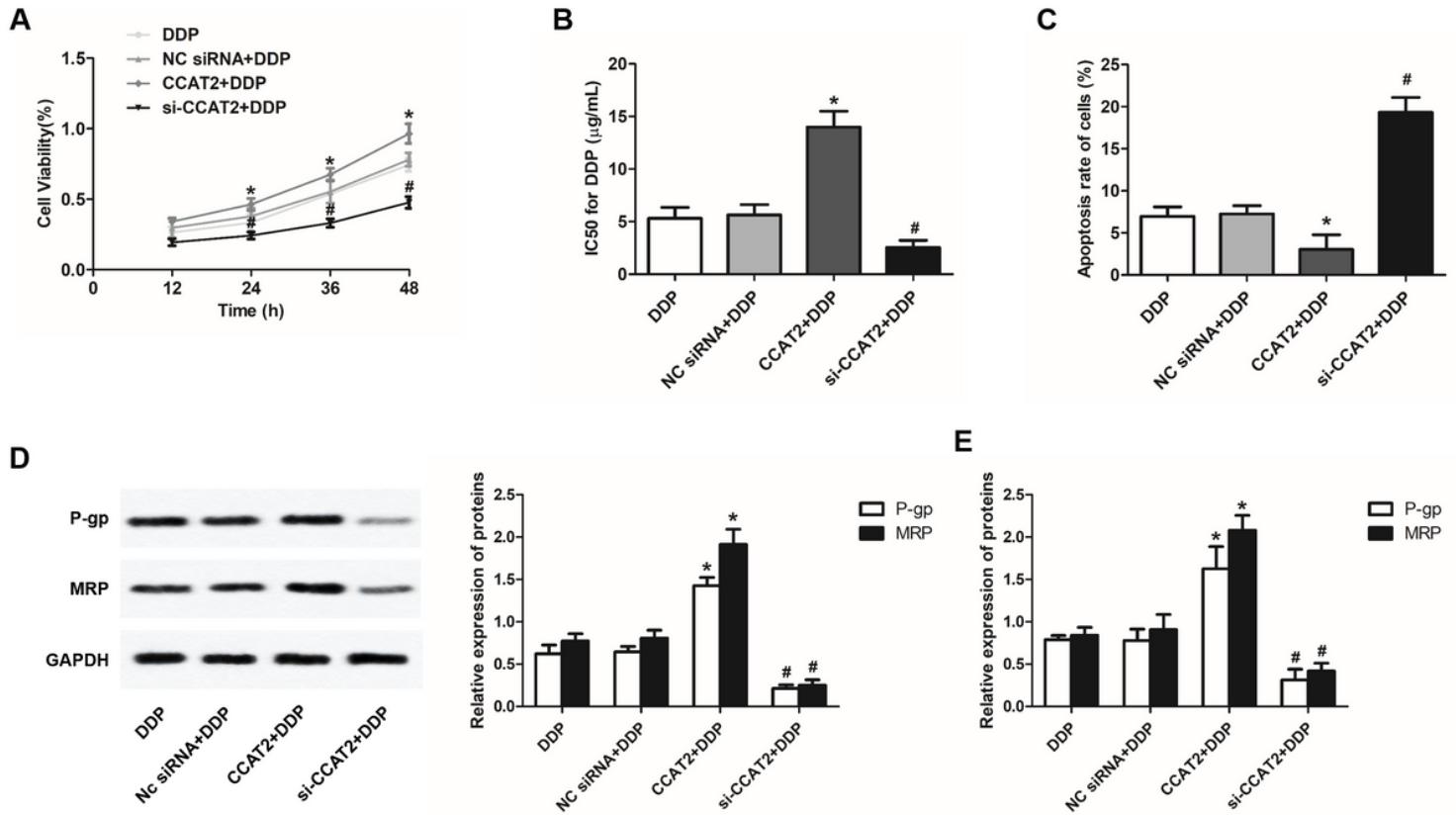
Figure 3

Aberrant overexpression of CCAT2 significantly decreased the sensitivity of A549 cells to the treatment of DDP. (A) Cell viability of A549 cells after treated by various strategies by CCK-8 assay. (B) IC50 values for DDP after received with different treatments. (C) Apoptosis of A549 cells at 24 h after treated by various strategies by propidium iodide and annexin V double stainingmethod. (D) The levels of P-gp and MRP in A549 cells, respectively treated with DDP, NC siRNA + DDP, CCAT2 + DDP, and si-CCAT2 + DDP, determined by Western-blot assay. (E) Quantitative investigation of the P-gp and MRP expressions in various strategies treated A549 cells by RT-qPCR assay. *P<0.05, significantly higher than the cells treated with DDP and/or NC siRNA + DDP. #P<0.05, significantly lower than the cells treated with DDP and/or NC siRNA + DDP.
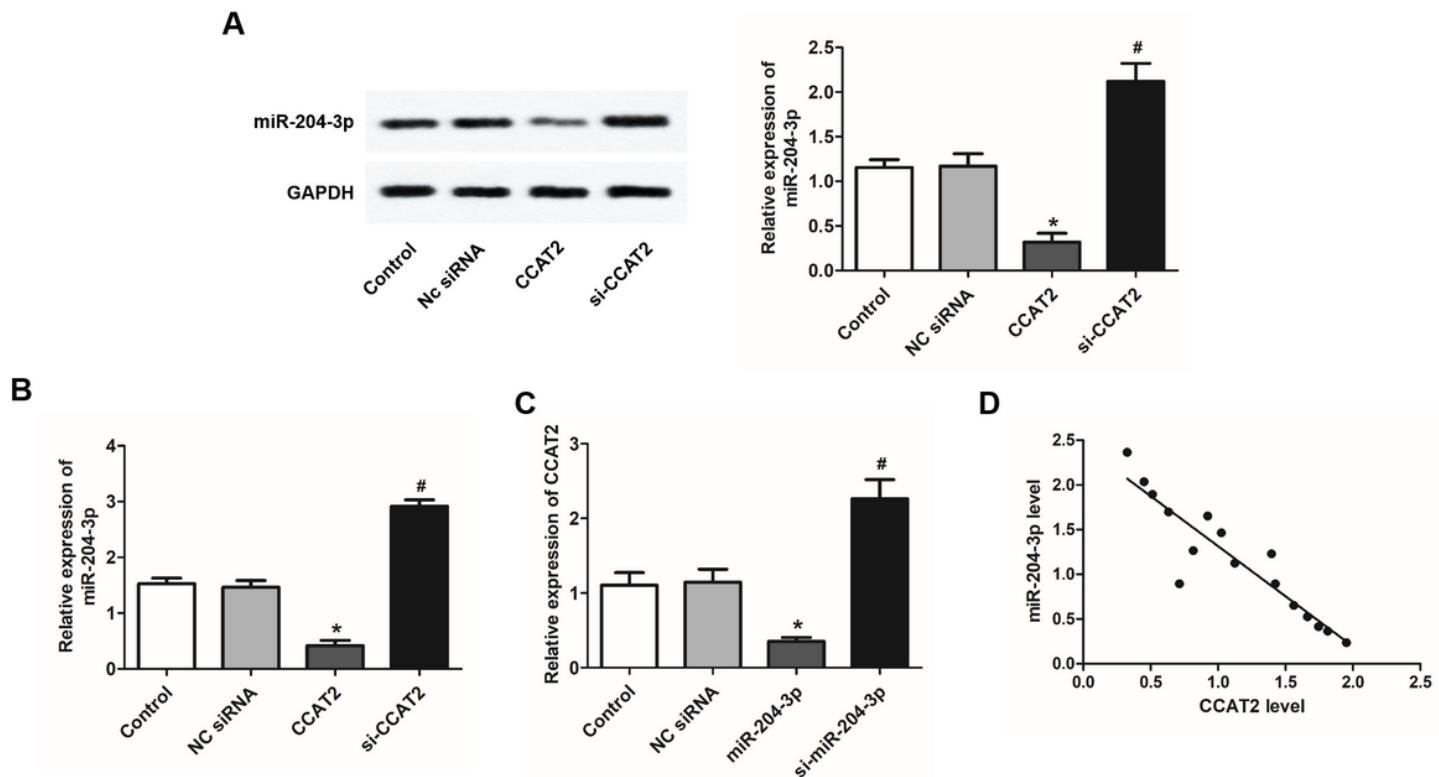
Figure 4

Increase the level of CCAT2 dramatically down-regulated the expression of miR-204-3p. (A) Qualitative analysis and semi-quantitative evaluation of the level of miR-204-3p in A549 cells after various treatments determined by Western-blot assay. (B) Quantitative investigation of the expression of miR-204-3p in A549 cells after different transfection examined by RT-qPCR assays. (C) RT-qPCR analysis of the expression of CCAT2 in A549 cells after respectively transfected with NC siRNA, miR-204-3p, and si-miR-204-3p. (D) Investigation of the correlation between the expression of miR-204-3p and CCAT2. *P<0.05, significantly lower than compared with the control group or cells treated with NC siRNA. #P<0.05, significantly higher than the control group or cells treated with NC siRNA.
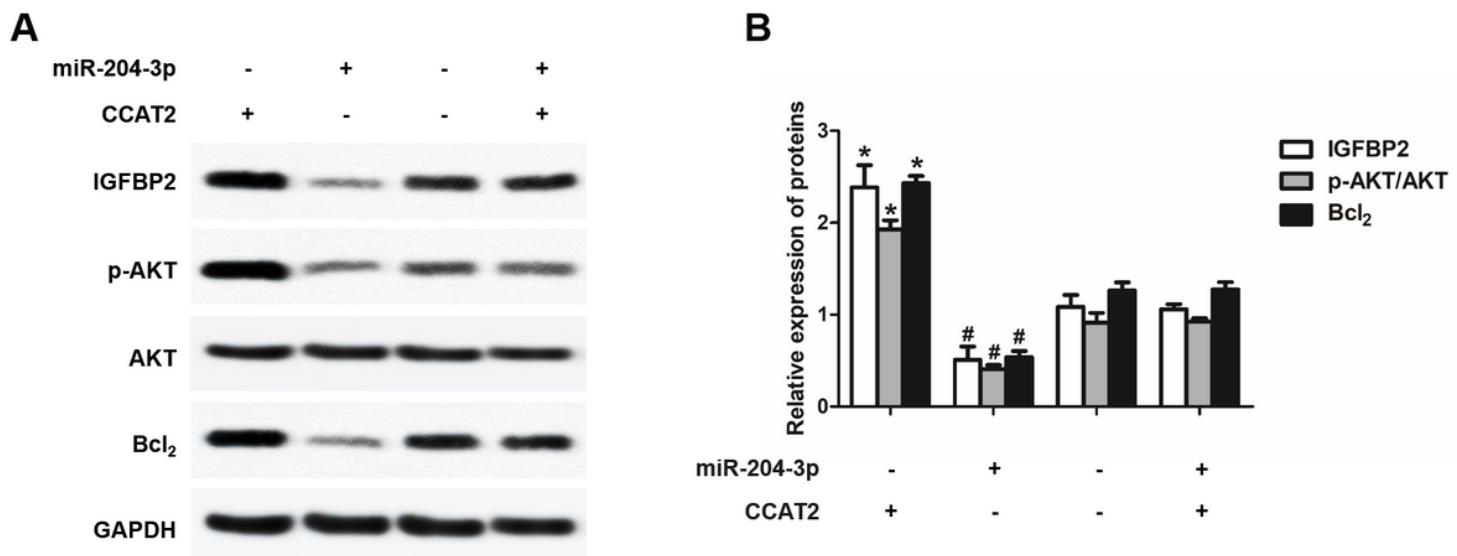
Figure 5

Increase the CCAT2 level signally inhibited the miR-204-3p mediated IGFBP2/AKT/Bcl2 pathway. Qualitative (A) and semi-quantitative (B) analysis of the downstream genes of miR-204-3p, including the IGFBP2, p-AKT/AKT, and BCl2, after different treatments. *P<0.05, significantly higher than the cells only transfected with miR-204-3p or CCAT2. #P<0.05, significantly lower than the cells only transfected with miR-204-3p or CCAT2.
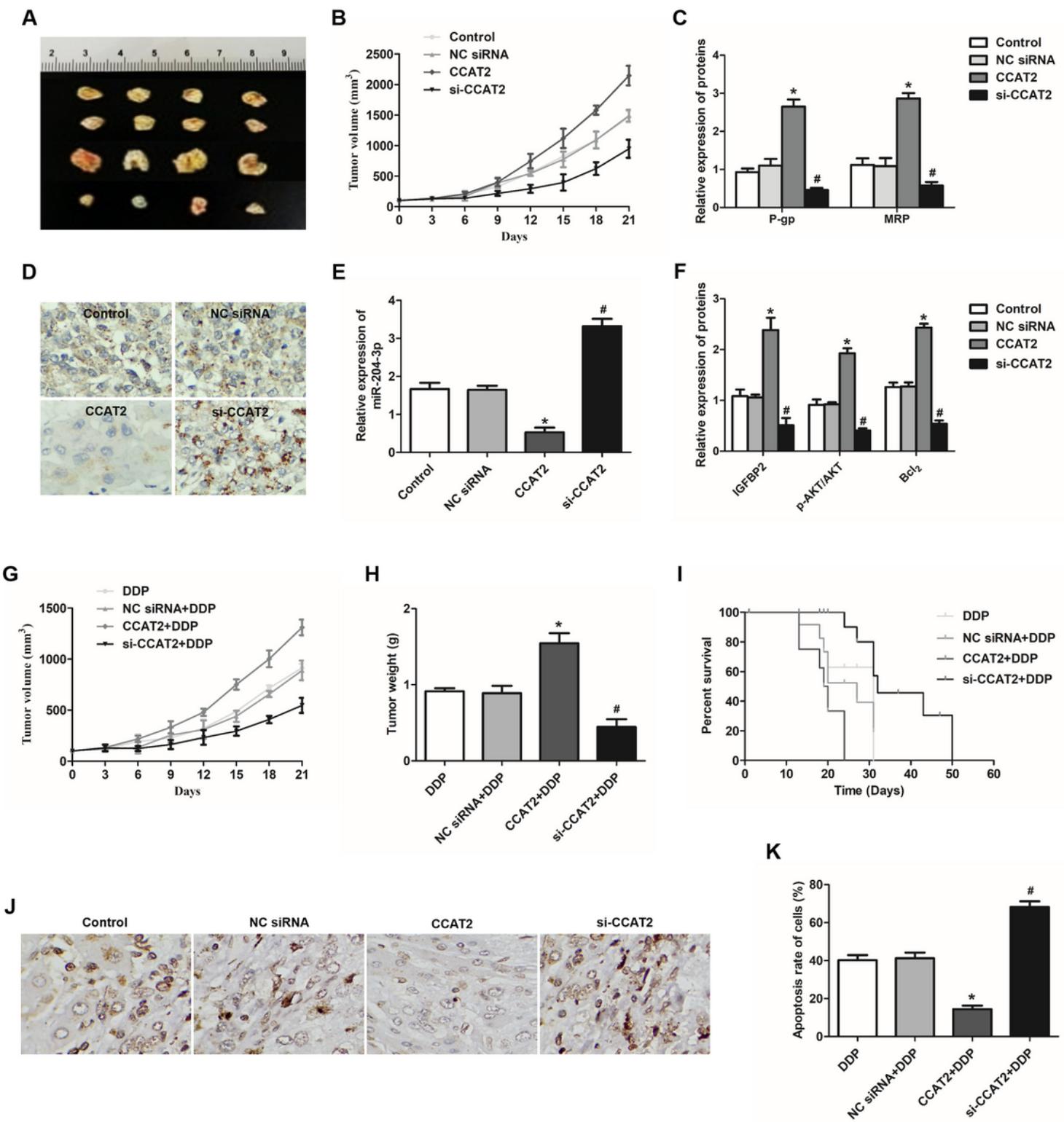
Figure 6

Overexpression of CCAT2 promoted the progress of A549 tumor thorough inhibition the miR-204-3p and activation of the IGFBP2/AKT/Bcl2 pathway. Tumor images (A) and tumor volumes changes (B) of mice after respectively treated by NC siRNA, CCAT2, and si-CCAT2. The mice transplanted with non-gene transfected A549 cells used as the control. (C) RT-qPCR analysis of the expression of P-gp and MRP in tumor tissues at the end of tumor growth experiments. Qualitative (D) and semi-quantitative (E) evaluation of the miR-204-3p level in tumor tissues by IHC analysis. (F) Quantitative analysis of the expression of IGFBP2, p-AKT/AKT, and Bcl2 in tumor tissues. Tumor volumes changes (G), tumor weight (H), and percent survival time (I) of mice after respectively treated by DDP, NC siRNA + DDP, CCAT2 + DDP, and si-CCAT2 + DDP. Qualitative (J) and semi-quantitative (K) evaluation of the cytotoxicity of DDP to tumor tissues by TUNEL assay. *$P<0.05$, significantly higher than the control group or cells treated with DDP, NC siRNA, or NC siRNA + DDP. #$P<0.05$, significantly lower than the control group or cells treated with DDP, NC siRNA, or NC siRNA + DDP.