

MicroRNA silencing of CNN1 and CNN2 protein family members regulates biology processes in colorectal cancer by targeting the p53 signal pathway

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Abstract

Background

Calponin was first defined as a striated muscle troponin T-like protein that binds actin thin filaments to regulate smooth muscle contraction. There are few studies of CNN1 and CNN2 in colorectal cancer, and the roles these two genes play in colorectal cancer cell lines and the mechanisms by which they act are unknown.

Methods

We used immunohistochemistry to identify expression of the two genes in the cancer tissues. RT-PCR was used to measure expression levels of microRNA. We performed western blots to measure changes in signaling pathways in the context of expression interference. Meanwhile, the same method was used to measure binding relationship between the two genes and key pathway proteins. To determine the relationship between microRNA and gene mRNA, we used the reporter gene method. We used the chi-square and t-test methods to analyze the significance and correlations of the data.

Results and conclusions

Expression levels of CNN1 were lower in colon cancer tissues than in normal mucosal tissues. After downregulating CNN1, the cell cycle in colon cancer cell lines progressed quickly, and the expression of related pathway proteins also increased. Expression levels of CNN2 were higher in colon cancer tissues, and its downregulation significantly inhibited cell cycle progression in colon cancer cell lines. We confirmed correlations between the expression of microRNA and CNN2 using data analysis. Bars indicate \pm standard errors. * $p < 0.05$; ** $p < 0.01$ compared with the control. The inhibition of the expression of CNN2 mRNA using microRNA was confirmed using western blot. The combination of the two at the mechanism level was also demonstrated using the reporter gene method.

Background

MicroRNAs play fundamental roles in regulation of several cancer, despite the fact that they are too small to code for proteins. Uses of microRNA in colorectal cancers include diagnosis, outcome prediction, and (in the future) therapeutic guidance [1]. Many investigators test expression levels of microRNA and its tissues, distinguishing cancer tissues from normal tissues; nevertheless, this may be sufficient to explain the underline mechanisms of microRNA in cancer progression [2]. In the present study, we adopted several approaches to determine how microRNAs participate in regulation of colorectal cancers as has been done in other cancers [3-7].

Calponin is a striated muscle troponin T-like protein that binding actin thin filaments to regulate smooth muscle contraction [8]. It is a protein of 34–37 kDa (292–330 amino acids), expressed in both smooth

muscle and many non-muscle cell types, where it functions as an inhibitor of actin-activated myosin ATPase [9].

We chose CNN1 (calponin isoform 1) and CNN2 (calponin isoform 2) to study calponin protein family members in colorectal cancer; these genes may turn out to regulate other cancers as well [10–11]. We used various methods and materials to demonstrated role of calponin protein family members in colorectal cancer cell lines.

Material And Methods

Patients and cell lines

We selected 200 patients with colorectal cancer from 2017 to 2019 at the Proctology Department, Affiliated Hospital of Youjiang. None of these patients received radiotherapy or chemotherapy prior to surgery. After the tumors were excised, they were sent to an independent pathology laboratory for testing. According to the international tumor guidelines, the tumors were staged, paraffin samples were prepared and stored in liquid nitrogen. The study was reviewed by the ethics committee on clinical trials and basic trial research of the proctology department at the Affiliated Hospital of Youjiang. The colorectal cancer cell lines and normal mucosal epithelial cell lines mentioned in this paper were purchased from Shanghai Cell Bank.

Cell culture and transfection

Five cell lines are selected to be cultured in RPMI 1640 and 20% fetal calf serum. After 3 to 4 passages, the cells were fully expanded and the density was uniform and not stacked. Cells grown in flasks were exposed to trypsin and seeded into six-well plates (Corning) to a density of about 50% prior to addition of transfection reagents. These reagents included two microRNA mimics and inhibitors, and two gene silencing reagents (iRNA), all of which are purchased from jima gene. The subsequent plasmids were purchased from OriGene, and the transfection reagent Lipo3000 was purchased from Thermo Fisher Scientific. After the cells are expanded to about 50% of the plate area, transfection reagents, including Lipo3000 and iRNA or plasmids, were added evenly. After culturing for 48 hours, the cells were collected into centrifuge tubes, stored frozen or used for machine detection. We chose the mimic and inhibitor of iRNA and microRNA in HCT-116 cells with high expression of both genes to explore the role of these two genes and microRNA. We then used the relatively low expression of HT29 cell line. A rescue experiment was carried out by upregulating the expression of genes using plasmids.

Immunohistochemistry

From the resected tumors of patients with colorectal cancer, thin paraffin sections were prepared, heated in a 70 °C oven for 2 hours, and then dewaxed (xylene-pure ethanol, 75% ethanol for 30 minutes each). Sections were processed according the instructions of the Maixin-Bio reagent immunohistochemistry kit. We added solution A (catalase) dropwise to eliminate non-atopic staining (30 minutes), rinsed with PBS

three times and incubated with solution B (Ultra V Block) for about 30 minutes, then added primary antibody, and stored at 4 °C overnight. The following day, we rinsed with PBS solution 3 times, incubated with solution C (Primary Antibody Enhancer) for about 15 minutes, rinsed with PBS solution 3 times, and incubated with solution D (enzyme labeled secondary antibody) for about 15 minutes. DAB chromogenic solution was stained for 5 minutes. Then concentrated hydrochloric acid was applied for 6 seconds, hematoxylin was stained for 5 minutes, and alkaline water was added to turn blue for 3 minutes. After final dehydration (75% ethanol-pure ethanol-xylene for 30 minutes each), the seals were retained.

We observed the stained sections under a light microscope. After selecting the field of view, according to the staining intensity, we classified the sections as follows: strong, 3 points; strong, 2 points; weak, 1 point; and none, 0 points. Evaluation of the range of staining was as follows: 100%–75%, 4 points; 74%–50%, 3 points; 49%–25%, 2 points; 24%–0%, 1 point. Those with total scores greater than or equal to 6 points were judged as showing strong expression.

Cell cycle

After 48 hours, the cells transfected with iRNA or plasmids and were trypsinized into centrifuge tubes, rinsed 3 times with PBS, and transferred to cell cycle staining solution (PI), protected from light at 37 °C for 30 minutes, and then placed in the cytometer for measurement. The results were analyzed using software. Cell cycle reagent stains were purchased from Thermo Fisher Scientific.

Western blot and co-immunoprecipitation

After culturing the cells that had been cultured for 48 hours in PBS, we washed them three times, and then incubated in RIPA for 30 minutes on ice. After centrifugation, we removed the supernatants and tested cells on a microplate reader to quantify them against protein standards. After configuring the electrophoresis gel, we dropped the samples into wells of the gel plate at the same volume (30 µl) one by one, and started the electrophoresis to the loading mark to the bottom of the gel plate, then cut the gel according to the markers and transferred them onto PVDF membranes.

Subsequently, the membranes were rinsed three times with TBST solution (Thermo Fisher Scientific), and the primary antibody was incubated overnight at 4 °C. After washing three times with TBST solution, rabbit secondary antibody was added and incubated at room temperature for 2 hours. After washing thoroughly, we added luminescent solution (Thermo Fisher Scientific) to an ECL luminometer (Bio-Rad) for measurement.

For co-immunoprecipitation experiments, luminescence measurement was performed as described above. For sample preparation, we used 30 µl of magnetic beads (Thermo Fisher Scientific) that had been bound to specific antibody in the supernatants after complete lysis, and then mixed on the machine and stored at 4 °C overnight. We discarded the supernatants and retained the magnetic beads. After washing the TBST solution, we add the loading solution and boiling water to separate the protein on the magnetic beads, and then performed protein electrophoresis according to the steps described above.

Real time PCR

TRIzol reagent (Takara Bio, Shiga, Japan) was used to isolate total RNA from frozen cancer tissues. We added 1 ml of TRIzol reagent and 300 μ l of chloroform to the tissue, mixed, and then centrifuged at 10,000 rpm for 15 minutes. Next, an equal volume of isopropanol was added to the supernatants, and the samples were centrifuged again at 10,000 rpm for 15 minutes. The supernatants were discarded and the precipitate was washed with 1 ml of 75% ethanol. Next, we reverse transcribed the extracted RNA according to the manufacturer's instructions (Takara). Then, we designed amplification primers for the messenger microRNA based on the specific sequence (Shanghai, China). The expression of mature microRNA was measured using the Dice real-time system II thermal cycler under the following conditions: 95 °C for 30 s; 45 cycles, 95 °C for 5 s; and 60 °C for 60 s; finally, we performed melting curve analysis. The relative expression of microRNA was normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase mRNA, and the fold-change in expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Compared with adjacent non-tumor tissues, we defined negative values as the relatively low expression of microRNA, and positive values as relatively high expression microRNA in colorectal cancer.

Dual-luciferase reporter assay

For the dual-luciferase reporter assay, the 3'-UTR of the CNN1 and CNN2 mRNA and mutant CNN1 and CNN2 mRNA were synthesized and inserted into pMIR-REPORT Luciferase plasmids (OBIO, Shanghai, China). The binding points of the wild-type CNN1 and CNN2 mRNA predicted using software and the microRNAs were identified in the figure below. Human 293 cells were cultured in 6-well plates, and when they were nearly 30% confluent, they were cotransfected with the pMIR-REPORT luciferase-CNN1, CNN2 (0.4 μ g) or pMIR-REPORT-MT-CNN1 and CNN2 (0.4 μ g) plasmid, the pRL-TK luciferase reporter (100 ng/well) and the microRNA-140-3p mimic (25 nmol/L) or mimic-NC (25 nmol/L) using Lipofectamine 3000 (Invitrogen). Twenty-four hours later, the cells were harvested, and luciferase activities were measured using a Varioskan Flash System (Thermo Fisher Scientific). Each experiment was performed three times.

Statistical analysis

Tumor-related information and immunohistochemical scores were evaluated using χ^2 tests, and expression levels of mRNA and protein were compared using the independent sample T test after obtaining quantitative data. P-values less than 0.05 were considered significant. All data analyses were performed using SPSS 17.0.

Results

High expression levels of CNN2 and low-expression levels of CNN1 were detected in colon cancer tissues

After sectioning, 200 paired colon cancer tissues were tested using immunohistochemistry. In 84 cancer tissues, there were higher expression levels of CNN1 than in others; 113 cancer tissues were characterized

by upregulated CNN2 expression with respect to with normal tissues, suggesting that CNN1 and CNN2 may play roles in development of colorectal cancer (Table 2, Figure 1). We also tested protein and RNA expression levels of the two genes in colorectal cancer cell lines and normal mucosal tissues (Figure 2). Our findings suggested a basis for a gene intervention study to select relevant cell lines.

Correlation between clinical data analysis or protein expression analysis and CNN1 and CNN2

Ki67 and p53 are cancer-associated proteins [12-15]. We used immunohistochemistry to measure Ki67 and p53 expression in colon cancer tissues (200 samples). We found that when CNN1 was expressed at lower levels in cancer tissues, Ki67 expression levels in colon cancer tissues were higher ($p = 0.002$), while levels of p53 expression were lower ($p = 0.000$). By contrast, when CNN2 was expressed at higher levels, expression levels of p53 in colon cancer tissues were lower ($p=0.002$), while Ki67 expression levels were higher ($p = 0.007$). The definitions of “high” and “low” are described above (Table 1, Figure 3).

Table 1. Correlation between p53 and Ki67 expression and CNN1 and CNN2 analysis

Parameter		CNN1		P value	CNN2		P-value
		low	high		low	high	
p53	Low	76	6	0.000	25	57	0.002
	High	40	78		62	56	
Ki67	Low	67	66	0.002	67	66	0.007
	High	49	18		20	47	

Next, we used statistical analysis to determine relationships between clinical data and CNN1 and CNN2 expression levels. We found a negative correlation of CNN1 expression with tumor size ($p = 0.000$) and T stage ($p = 0.006$). For CNN2, only T stage ($p = 0.002$) was positively related with high expression levels of CNN2. No other clinical data showed significance with respect to CNN1 or CNN2 expression levels (Table 2).

Table 2. Clinical data analysis with CNN1 and CNN2 expression

Parameter		CNN1		P value	CNN2		P-value
		low	high		low	high	
Age (year)	< 65	58	37	0.474	36	59	0.154
	≥65	58	47		51	54	
Gender	Male	59	39	0.568	40	58	0.478
	female	57	45		47	55	
T stage	1	6	15	0.006	12	9	0.002
	2	27	27		29	25	
	3	56	29		39	46	
	4	27	13		7	33	
N stage	0	35	27	0.331	26	36	0.820
	1	54	31		36	49	
	2	27	26		25	28	
M stage	0	55	38	0.872	41	52	0.887
	1	61	46		46	61	
Differentiation	poor	24	22	0.612	17	29	0.573
	medium	43	27		31	39	
	well	49	35		39	45	
Tumor size(cm)	< 5	34	60	0.000	42	52	0.776
	≥5	82	24		45	61	

Expression of upstream microRNA filtered has fundamental significance for CNN1 and CNN2 expression

Filtered from data groups, mir-939-5p and mir-4492 were identified using PCR. All 200 paired colon cancer tissue expression levels of mir-939-5p and mir-4492 were compared with CNN1 and CNN2 protein expression (defined above). Mir-939-5p and mir-4492 expression negatively correlated with CNN1 expression. Mir-939-5p and mir-4492 expression significantly negatively correlated with CNN2 expression (Table 3). These findings suggest that mir-939-5p and Mir-4492 may participate in upstream regulation CNN1 and CNN2 in colon cancer.

Table 3. MiRNA analysis of CNN1 and CNN2 expression

Parameter		Mir-939-5p			Mir-4492		
		t	$X \pm \sigma$	P value	t	$X \pm \sigma$	P-value
CNN1	LOW	-4.285	-0.695±4.824	0.000	-5.843	-1.808±5.977	0.000
	HIGH		2.205±4.582			2.868±4.996	
CNN2	LOW	2.771	1.605±4.548	0.006	3.483	1.803±5.976	0.001
	HIGH		-0.310±5.061			-1.113±5.791	

Regulation efficiency detection in colon cell lines

All colon cell lines were tested using PCR and western blot. Mir-939-5p, mir-4492, CNN1 and CNN2 expression are shown in Figure. The genes we identified were highly expressed in the HCT-116 colon cancer cell line, so we chose to conduct interference experiments in this cell line to downregulate protein expression of the two genes. MicroRNA interferes with gene transcription and thereby affects protein expression; for this reason, we also chose to conduct the microRNA interference test in the HCT-116 cell line that showed high gene expression. The SW480 cell line showed lower protein expression levels; therefore, we performed the rescue test using by plasmids in this cell line. We measured gene expression in these cell lines after iRNA interference using western blot (Figure 4).

MicroRNA (negative) and CNN1 (negative) or CNN2(positive) regulate colon cancer cell lines via the cell cycle

Mir-939-5p and mir-4492 mimics, added to cancer cell lines, arrested the cell cycle and decreased the speed of growth. Conversely, mir-939-5p and mir-4492 inhibitors promoted colon cancer cell lines in terms of the cell cycle and accelerated the speed of growth. Using CNN2-silencing iRNA, we tested the results of microRNA mimics. CNN1 silencing promoted colon cancer cell cycle progression. Taken together, these results suggest that CNN2 promoted cancer progress, while microRNA and CNN1 had the opposite effect (Figure 5).

MicroRNAs regulate p53 and Ki67 expression in cell lines through CNN1 and CNN2

Expression levels of p53 were upregulated after silencing CNN2, while Ki67 was downregulated, suggesting that CNN2 may be upstream of p53 and Ki67. When we knocked out CNN1 alone, we obtained the opposite result. We found that mir-939-5p and mir-4492 mimics decreased expression levels of CNN2 and Ki67; p53 expression was enhanced. Using mir-939-5p and mir-4492 inhibitors, we obtained the opposite results as seen on western blot (Figure 6).

RAS signal pathways are also activated by regulated CNN1 and CNN2

RAS is a fundamental protein in colon cancer [16-18]. Silencing CNN2 downregulated expression levels of K-ras and cyclin D1 proteins. The same results were obtained with mir-939-5p and mir-4492 mimics regulation. Mir-939-5p and mir-4492 inhibitors reversed K-ras and cyclin D1 expression in colon cancer cell lines. While silencing CNN1, we found that K-ras expression was highly stimulated (Figure 6).

CNN2 plasmids reverse the effect of microRNA

CNN1 and CNN2 plasmids were purchased from OriGene. We added plasmids (CNN1 and CNN2) and microRNA mimics together to interact with colon cancer cell lines. We found that plasmids (CNN2) both reversed the effects of mir-939-5p and mir-4492 mimics (Figures 4, 7).

Mir-939-5p and Mir-4492 regulates colon cancer cells by interacting CNN2, which targets the p53 signal pathway directly

Fluorescein reporter genes were used to determine the relationship between mir-939-5p and mir-4492 and proteins (CNN1 and CNN2). We found that mir-939-5p and Mir-4492 downregulated the luciferase signal (CNN2), while CNN1 was not suppressed, suggesting that mir-939-5p and mir-4492 regulate CNN2 protein expression directly (Figure 8). We also wanted to know the downstream proteins, with which CNN1 and CNN2 interacted in colon cancer cell lines. To accomplish this, we used co-immunoprecipitation to measure the relationship between p53 and K-ras and CNN1 and CNN2 (Figure 9). CNN2 co-immunoprecipitated with p53 and Ras proteins. These results suggest that mir-939-5p and mir-4492 interact in colon cancer cell lines via CNN2, and that CNN1 and CNN2 regulate cell lines by targeting the p53 signal pathway.

Conclusion

In this experiment, we explored the mechanism of action of two MicroRNA and CNN1 and CNN2 in intestinal cancer cell lines and patient cancer tissues. CNN1 acts as a factor that inhibits cancer development, inhibits intracellular P53 signaling pathway and regulates colorectal cancer cell lines. Cycle progress. CNN2, as an important protein that promotes the development of cancer, promotes the cycle progression of colorectal cancer cell lines. MicroRNA-939-5p and MicroRNA-4492 can directly regulate the expression level of CNN2 protein mRNA, thereby triggering the signaling pathway and cycle changes of colorectal cancer cell lines.

Discussion

Colorectal cancer is the third most common cancer in men and second most common cancer in women worldwide [19]. With earlier testing and systematic treatment, 90% patients with colorectal cancer can be prevented [20]. In the context of such research, we hope to contribute to the diagnosis of colorectal cancer. Because microRNA and calponin have been studied in cancer tissues before, we turned our attention to the underlying stimuli that interact with protein signal pathways and guide diagnosis and prediction of colorectal cancer.

CNN1 suppresses several cancers, including ovarian cancer, by interfering with initiation and progression [10, 21]. In hepatocellular carcinoma cells, CNN1 inhibited invasion and migration, suggesting that CNN1 suppresses several solid tumors [22]. In the present study, we found that CNN1 inhibits growth of colorectal cancer cell lines by interacting with the p53 and Ras signal pathways. CNN1 silencing also promoted cell cycle progression in colorectal cancer, consistent with findings of previous CNN1 studies in other solid tumors.

CNN2 is an actin filament-associated regulatory protein that is closely associated with cell proliferation and migration [23, 24]. As reported previously, when regulated by microRNA-296-5p, CNN2 interacted with progression of hepatocellular carcinoma cell lines. CNN2 silencing in gastric cancer arrested cancer growth [25]. As in previous studies, we found that CNN2 silencing inhibited cell cycle progression, and downregulate the p53 and RAS signaling pathways. We found that microRNA-939-5p and microRNA-4492 slightly downregulated CNN2 expression and the effect of CNN2. To demonstrate the direct targeting of microRNA to CNN2, we conducted a rescue experiment, and found that the CNN2 plasmid strongly reversed the effect of microRNA.

It was reported that lncRNA LINC00460 promoted colorectal cancer cells metastasis via microRNA-939-5p sponging [26]. We found that microRNA-4492 suppressed several cancer tissues as in previous studies. In the present study, we found that microRNA-939-5p and microRNA-4492 slightly downregulated CNN2 expression and progression.

Cyclin D1, a cell cycle promoter, was downregulated by silencing CNN1 and CNN2, explaining that why cell cycle was arrested [27, 28]. The K-ras and p53 signal pathways play important roles in colorectal cancer cell lines, as reported in many solid cancers [29–34]. We found that the p53 signal pathway was suppressed by upregulating CNN2 or silencing CNN1, which interacted with cyclin D1, arresting the cell cycle. The RAS signal pathway was activated by upregulating CNN2 or silencing CNN1, which stimulated the ERK-related signal pathway, promoting proliferation of colorectal cancer cell lines. Taken together, these results suggest that micro-939-5p and microRNA-4492 may suppress proliferation of colorectal cancer cell lines by targeting CNN2, and by interacting with the p53 and RAS signal pathways.

Declarations

Ethics approval and consent to participate

The Ethics Committee of the Proctology Department, Affiliated Hospital of Youjiang approved the research project and analysis of the patient specimens for this study.

Consent for publication

Not applicable

Availability of data and materials

Please contact the corresponding author for data requests.

Funding

This study did not receive any funding.

Competing interests

The authors have no competing interests.

Informed consent

All patients have signed an informed surgical consent before surgery. Informed consent includes the possibility of using patient specimens for clinical trial research, and promises to keep patient information and privacy completely confidential.

Author' Contribution

The researchers have a clear division of labor and outstanding contributions. As co-first authors, fuda wang and weiming wei participated in the experiment design and thought guidance, and anmin wang, ya zhang, zebang qin, and yi wu participated in the experiment implementation and data collection. Hua li and junyu gou participated in the writing and revision of the article. As co-corresponding authors, jian pu and houji guo participated in the overall arrangement and funding of the entire experimental idea.

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Figures

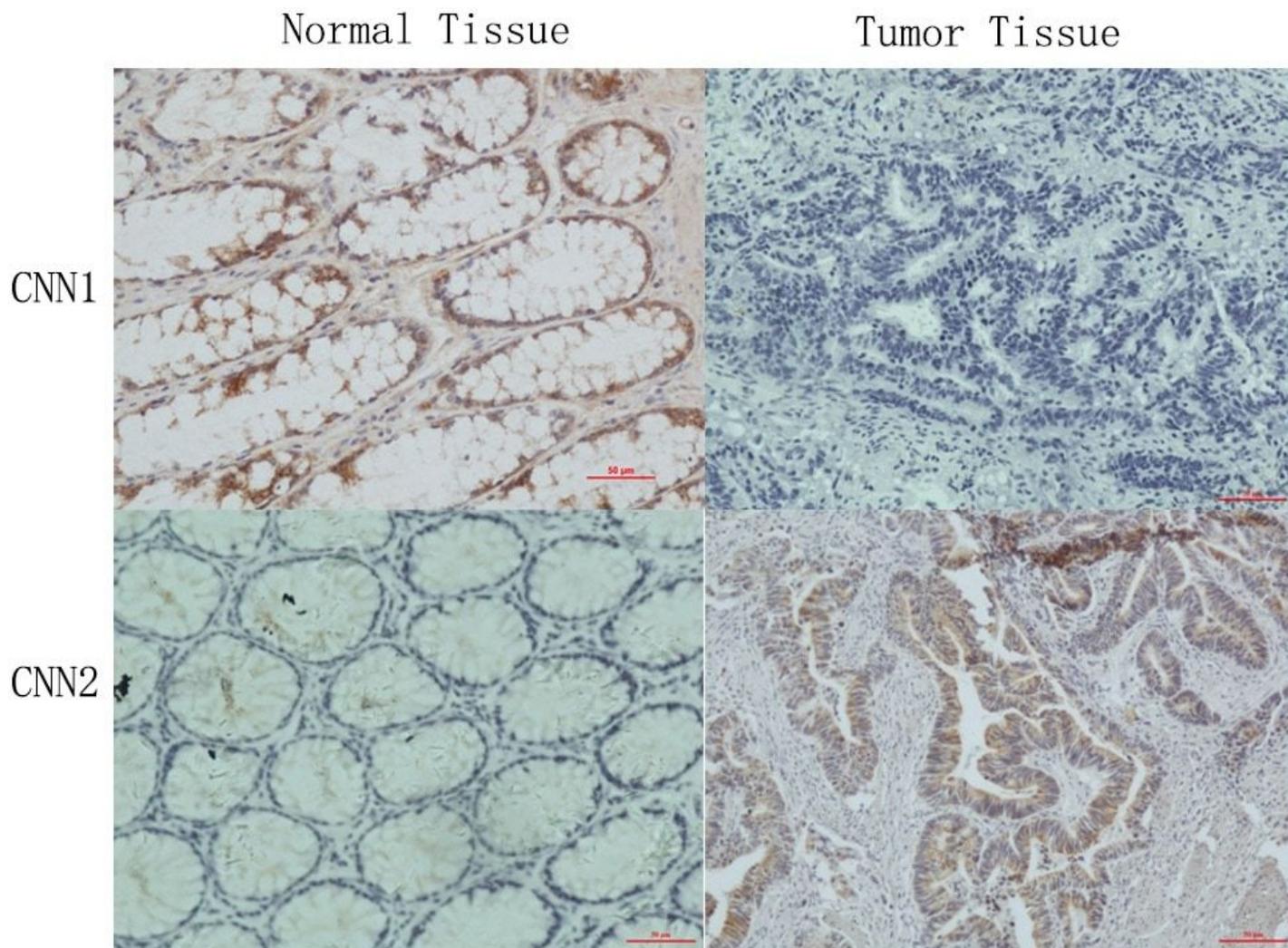


Figure 1

High expression levels of CNN2 and low-expression levels of CNN1 were detected in colon cancer tissues. Expression of CNN1 and CNN2 in normal colon mucosa tissue and corresponding colon cancer tissue. CNN1 is highly expressed in normal mucosa tissue and CNN2 is highly expressed in colon cancer.

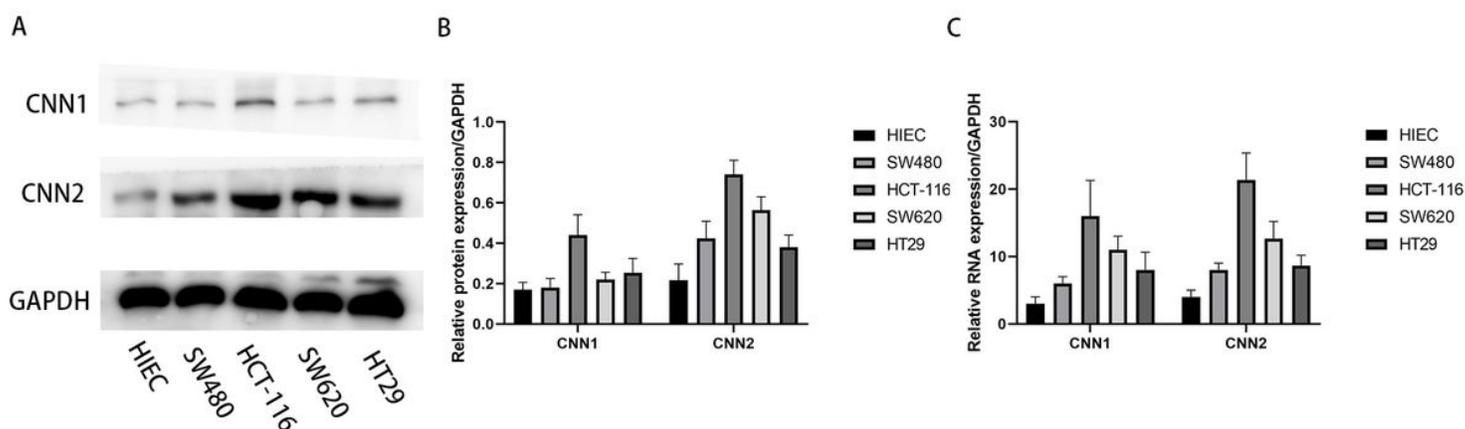


Figure 2

Correlation between clinical data analysis or protein expression analysis and CNN1 and CNN2 A. Expression of CNN1 and CNN2 in four colorectal cancer cell lines, compared to the normal colon mucosal tissue cell HIEC cell line. B. data analysis. Bars indicate \pm standard errors. * $p < 0.05$; ** $p < 0.01$ compared with the control. C. mRNA expression levels of CNN1 and CNN2 in these five cell lines.

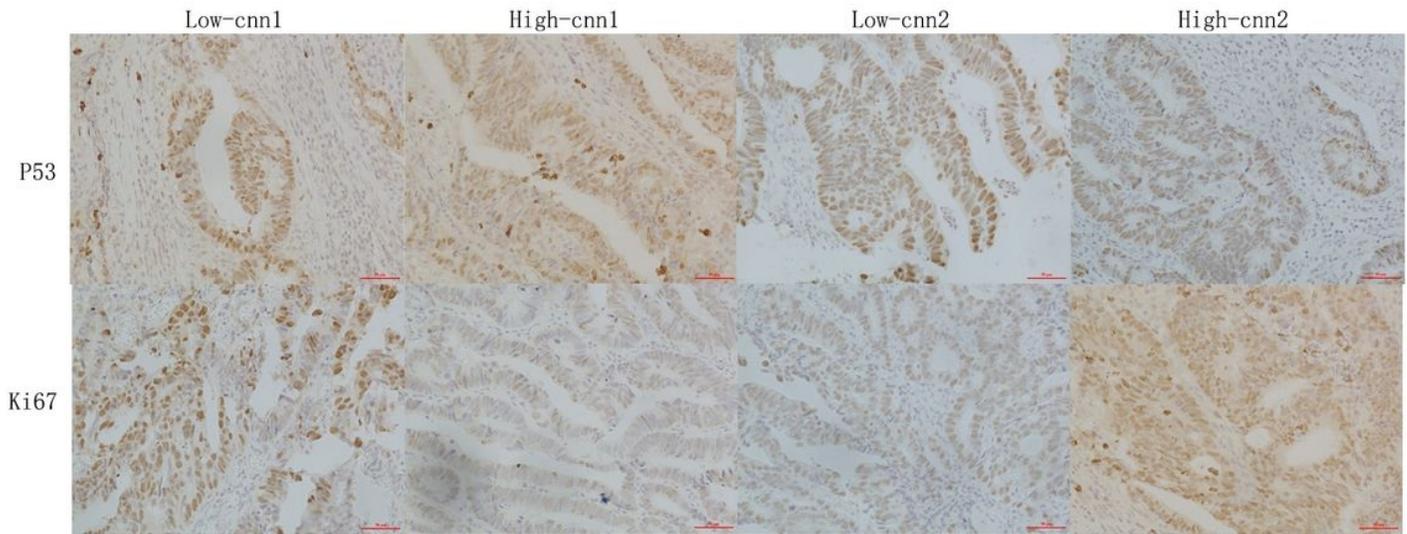


Figure 3

Expression of upstream microRNA have fundamental significance for CNN1 and CNN2 expression The results of immunohistochemistry after colon cancer tissue sectioning of the eight patients, revealing that Ki67 expression is positively correlated with CNN2 and negatively correlated with CNN1; p53 expression is positively correlated with CNN1 and negatively correlated with CNN2. After all the organization results are scored, the correlation analysis was completed and results are displayed in the table.

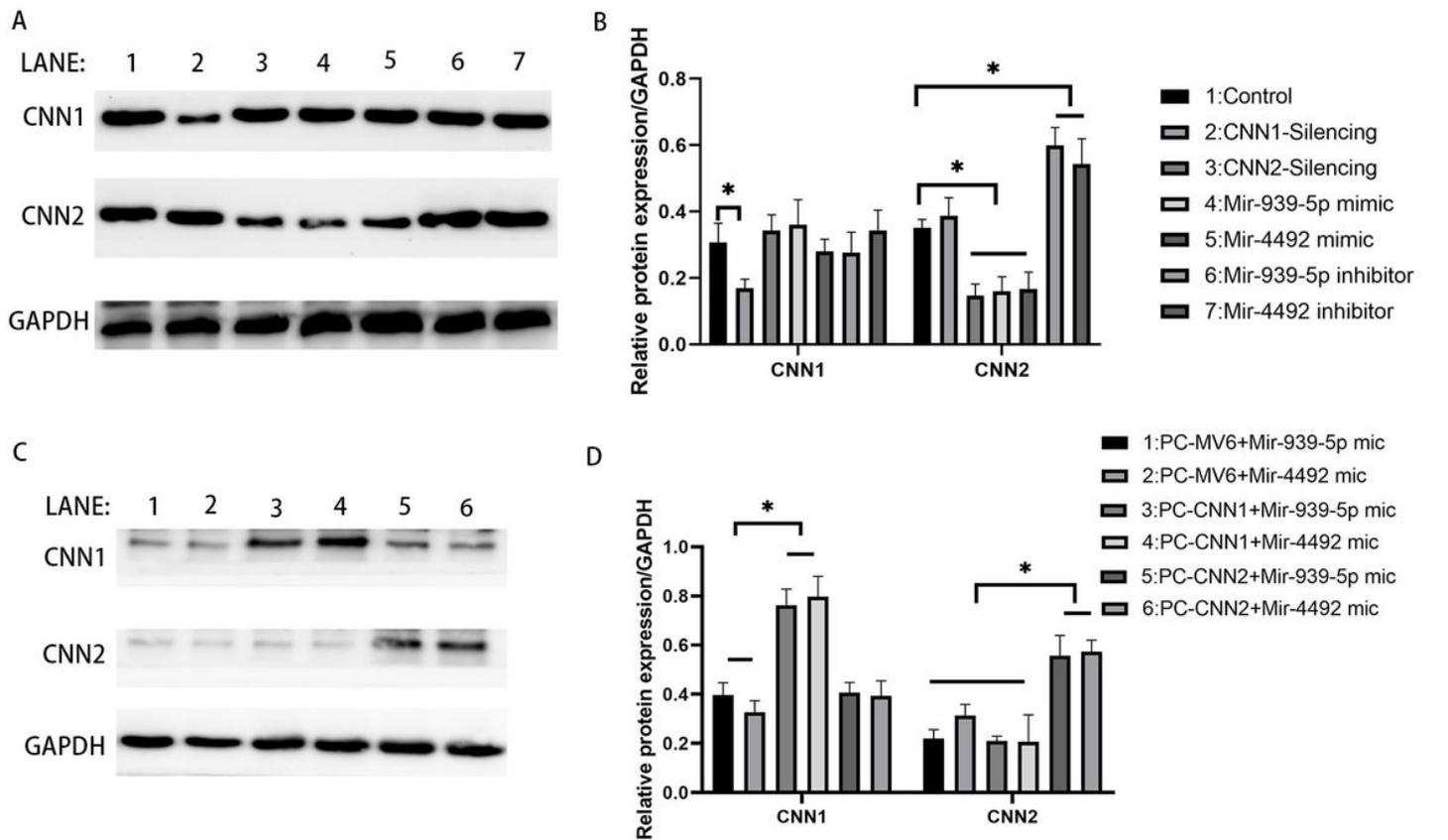


Figure 4

Regulation efficiency detection in colon cell lines A. After selecting the HCT-116 cell line as a carrier of interfering factors, the cells were lysed after 48 hours of culture and protein expression levels of CNN1 and CNN2 were measured. C. CNN1 and CNN2 protein expression levels were measured after HT29 cell line was used to upregulate plasmid genes. B, D. data analysis. Bars indicate \pm standard errors. * $p < 0.05$; ** $p < 0.01$ compared with the control.

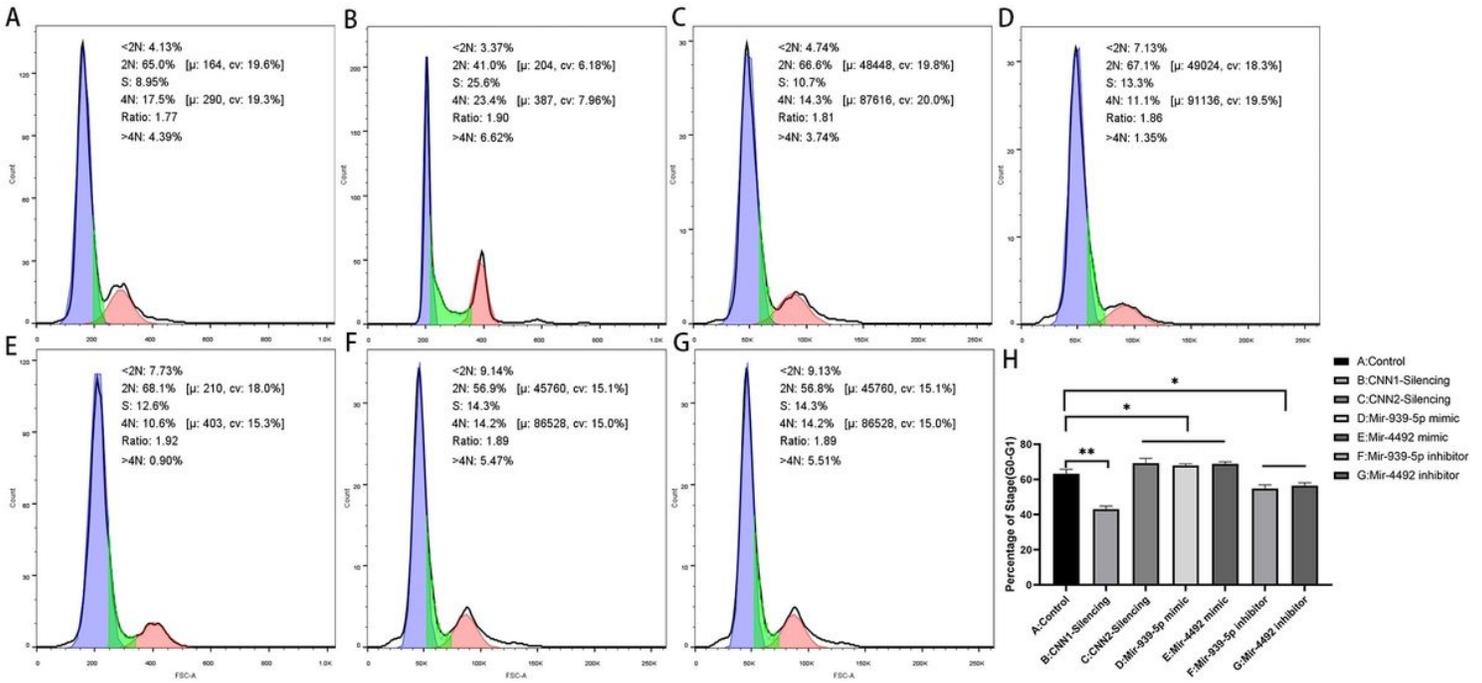


Figure 5

MicroRNA (negative) and CNN1 (negative) or CNN2 (positive) could regulate colon cancer cell lines by cell cycle. A–G. After interference of HCT-116 cell line, we found that the silencing CNN1 significantly promoted cell cycle progress. Silencing CNN2 and interference by microRNA mimic inhibited the cell cycle. MicroRNA inhibitor advanced cellular processes. H. data analysis. Bars indicate \pm standard errors. * $p < 0.05$; ** $p < 0.01$ compared with the control.

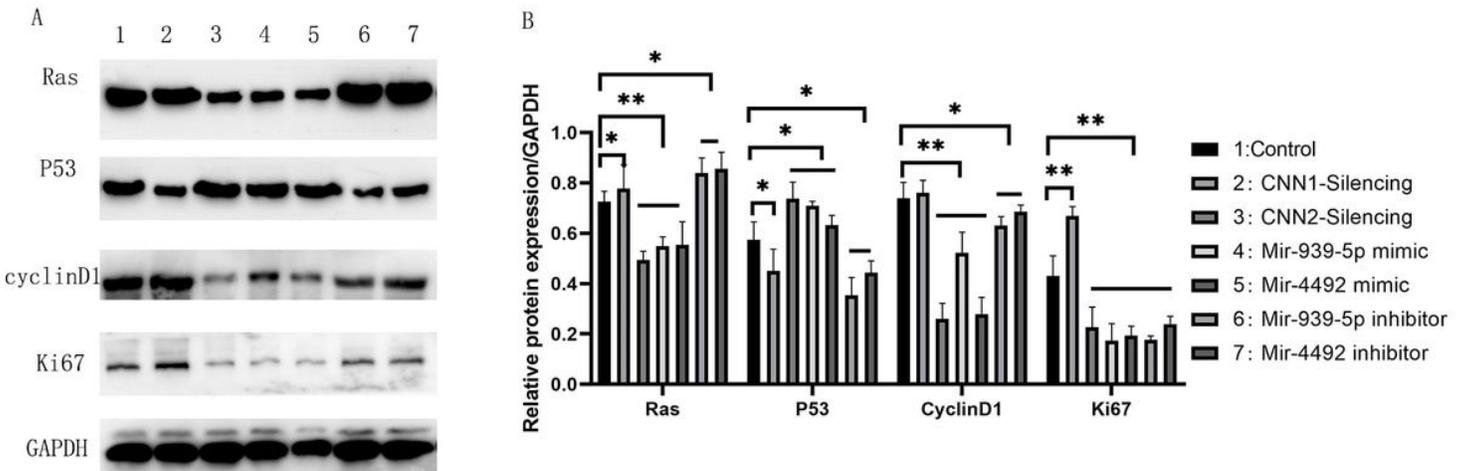


Figure 6

RAS signal pathways are also activated by regulated CNN1 and CNN2. A. After HCT-116 cell interference, western blot was used to measure key proteins in cell-related signaling pathways. CNN1 silencing and MicroRNA inhibitor inhibited the p53 signaling pathway, which enhanced expression levels of Ki67, while

CNN2 silencing and microRNA interference using mimic obtained the opposite result. B. data analysis. Bars indicate \pm standard errors. * $p < 0.05$; ** $p < 0.01$ compared with the control.

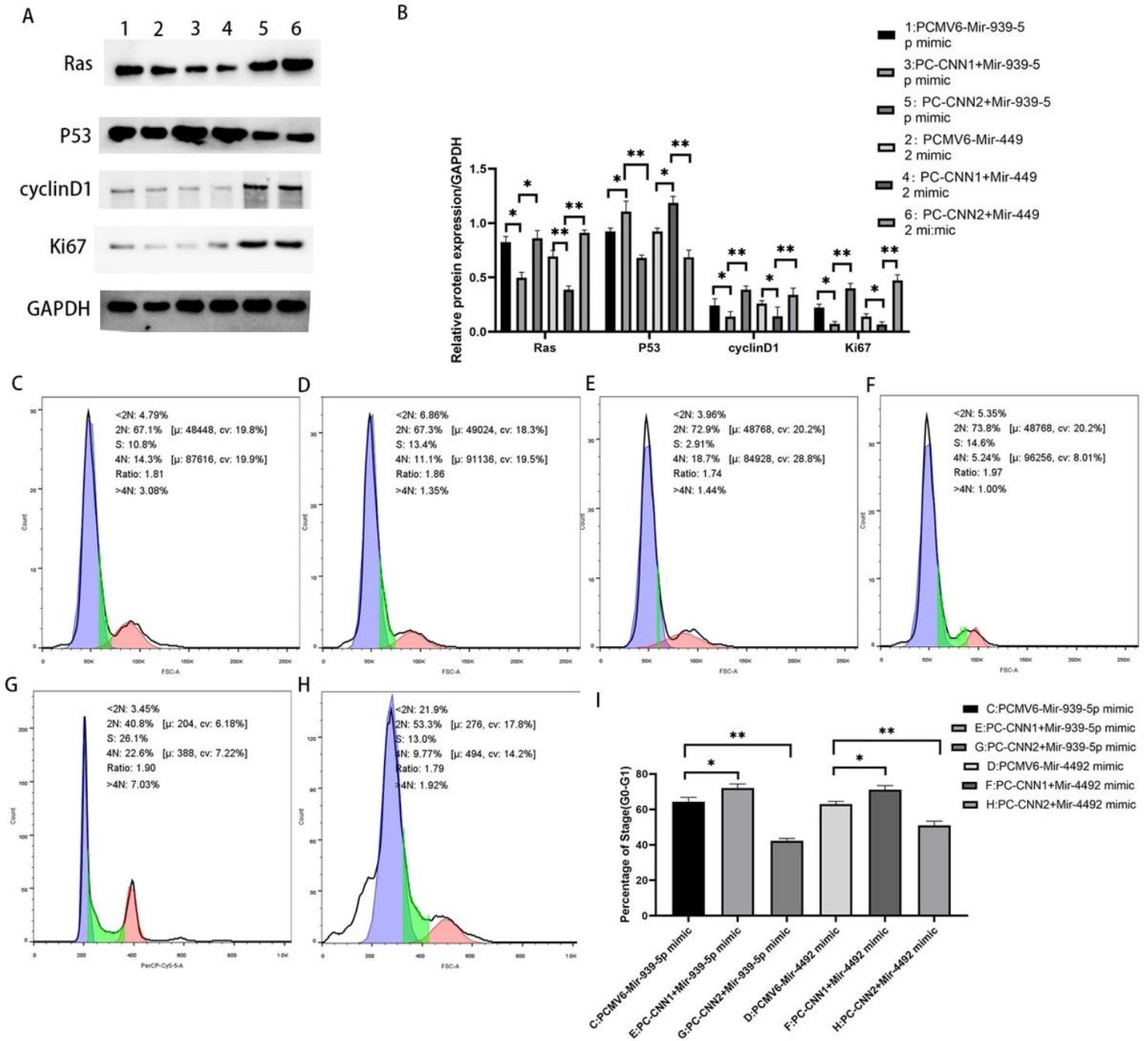


Figure 7

CNN2 plasmids reverse the effect of microRNA A. After double interference, we used HT29 cells to measure expression levels of relevant proteins. CNN1 plasmids significantly inhibited the Ras signaling pathway and relevant proteins and activated the p53 signaling pathway. The addition of CNN2 plasmids significantly promoted the activation of the Ras signaling pathway and increased expression levels of related proteins while inhibiting the p53 signaling pathway. B. data analysis. Bars indicate \pm standard errors. * $p < 0.05$; ** $p < 0.01$ compared with the control. C–H. After the HT29 cell line interfered with the cell

cycle, CNN1 inhibited cell cycle progression, and CNN2 promoted cell cycle progression. I. data analysis. Bars indicate \pm standard errors. * $p < 0.05$; ** $p < 0.01$ compared with the control.

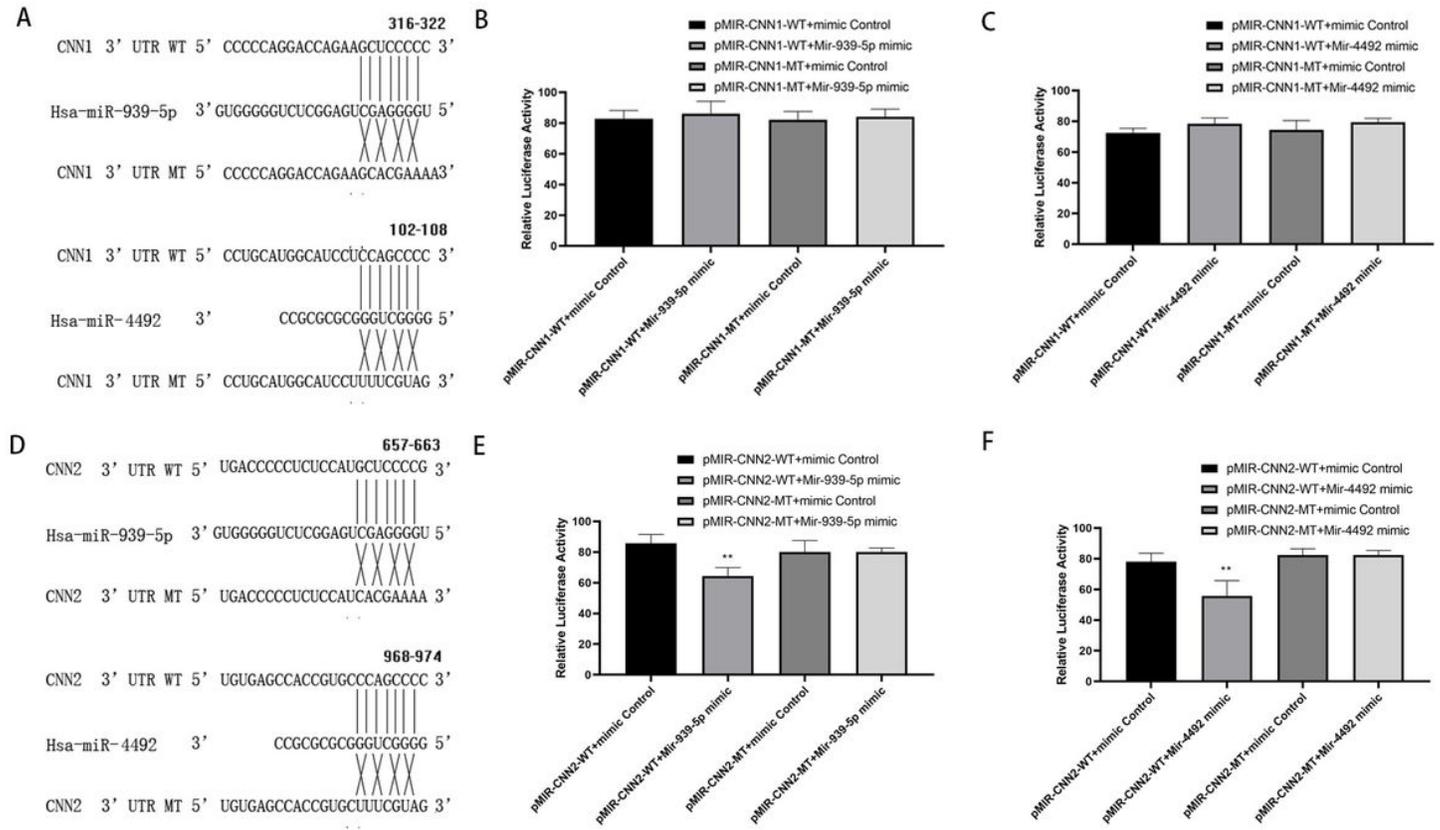


Figure 8

Mir-939-5p and Mir-4492 regulated colon cancer cells by interacting CNN2, which targets the p53 signal pathway directly. A, D software predicts the possible binding sites of mRNA of CNN1 and CNN2 genes and two microRNA. We used reporter genes to determine the possibility of binding, and found that microRNA-939-5p and microRNA 4492 can be combined with CNN2 mRNA binding. B, C, E, F. data analysis. Bars indicate \pm standard errors. * $p < 0.05$; ** $p < 0.01$ compared with the control.

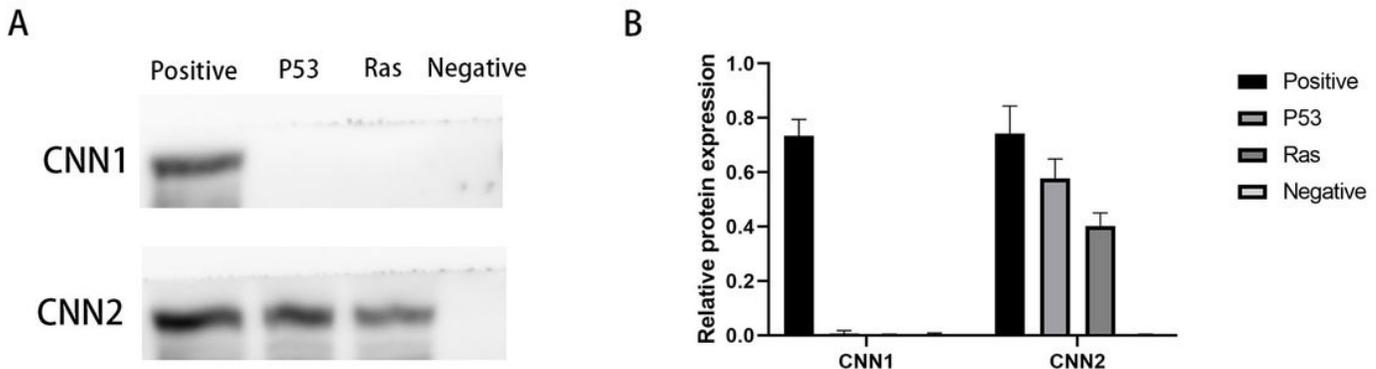


Figure 9

Mir-939-5p and Mir-4492 regulates colon cancer cells by interacting with CNN2, which targets the p53 signal pathway directly. A. HCT-116 cell line with relatively high expression was selected as the carrier for the co-immunoprecipitation experiment. CNN2 physically bound to p53 and Ras proteins. CNN1 did not give a positive result. B. data analysis. Bars indicate \pm standard errors. * $p < 0.05$; ** $p < 0.01$ compared with the control.