



NUP210 and MicroRNA-22 Modulate Fas to Elicit HeLa Cell Cycle Arrest

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Purpose: Cervical cancer is one of the most fatal diseases among women in under-developed countries. To improve cervical cancer treatment, discovery of new targets is needed. In this study, we investigated the expression of NUP210, miR-22, and Fas in cervical cancer tissues and their functions in cell cycle regulation.

Materials and Methods: We detected and compared the expression levels of NUP210, miR-22, and Fas in cervical cancer tissues with paired normal tissues using immunohistochemistry, Western blot, and real-time quantitative polymerase chain reaction. NUP210 was knocked down in HeLa cells via lentivirus, followed by cell cycle and proliferation analysis. Using a luciferase reporter assay, we explored the link between miR-22 and NUP210. We overexpressed miR-22 in HeLa cells and analyzed cell cycle and proliferation function. We then overexpressed miR-22 in NUP210 knockdown cells to explore the connection between Fas and miR-22-NUP210 signaling.

Results: We found that NUP210 was overexpressed in cervical cancer patients. Knocking down NUP210 restored cell apoptosis and proliferation. We confirmed miR-22 as a regulator of NUP210 and verified that miR-22 was inhibited in cervical cancer development. We also found that restoring miR-22 expression could induce cell apoptosis. Finally, we found that miR-22-regulated expression of NUP210 could alter Fas expression and, in turn, elicit cell cycle arrest and proliferation.

Conclusion: miR-22 in cervical cancer is downregulated, resulting in NUP210 overexpression and inhibition of Fas-induced cell apoptosis.

Key Words: Cervical cancer, apoptosis, cell cycle arrest, Fas, miR-22, NUP210

INTRODUCTION

Cervical cancer is one of the most life-threatening diseases among women, and despite vaccines against the human papilloma virus (HPV), which causes cervical cancer, it remains the third leading cause of female cancer-related death.¹ According

to the World Health Organization, in 2018, there were 570000 newly diagnosed cases of cervical cancer in under-developed countries, accounting for 84% of all cases worldwide, making it the second most common cancer in women in these countries (https://www.who.int/health-topics/cervical-cancer#tab=tab_1). Meanwhile, 311000 cervical cancer patients died in 2018, with over 85% from developing countries.

Despite the development of a vaccine, vaccination is not globally applied. Studies on public awareness involving HPV and its vaccine have indicated that awareness of HPV prevention and vaccination are significantly low, as indicated by the high frequency of cervical cancers in China.¹⁻⁶ High frequency rates and low public awareness highlight a major need for investigation into the mechanisms by which cervical cancer develops to inform the design of improved therapeutics.

NUP210 is a major component of the nuclear pore complex that is involved in regulating the transport of macromolecules

Received: January 13, 2020 **Revised:** March 11, 2020

Accepted: March 26, 2020

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•The authors have no potential conflicts of interest to disclose.

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between the nucleus and cytoplasm. The specific function of NUP210 has not been well characterized in many cancer models. However, studies have shown that NUP210 has a role in regulating cell fate, including cell differentiation.^{7,8}

MicroRNA-22 (miRNA-22, miR-22, and miR-22-3p) is a non-coding RNA that may bind to NUP210 mRNA and regulate its expression. miR-22 is a well-studied miRNA: in patients with pancreatic cancer, schizophrenia, or chronic heart failure, miR-22 is aberrantly expressed and considered a diagnostic biomarker.⁹⁻¹¹ In hepatocellular carcinoma, miR-22 suppresses cell proliferation by targeting Sp1,¹² demonstrating its inhibitory function. In addition, by targeting WRNIP1, miR-22 enhances the radiosensitivity of small-cell lung cancer,¹³ suggesting that it could be used as a novel treatment target. Moreover, in cervical cancer, miR-22 has been identified as prognostic, diagnostic, and therapeutic biomarkers.^{14,15} We, therefore, sought to examine the relationship between this miRNA and NUP210.

Research has shown that NUP210 expression is regulated by miR-22 and is closely related to Fas expression.^{16,17} Fas and its ligand, FasL, are members of the tumor necrosis factor family. The Fas-FasL system regulates cell death, and in cancer development, its deregulation is closely related to the immune escape of the tumor.¹⁸ In breast cancer, defective Fas-FasL has been found to cause aggressive tumor development, as Fas promotes cell death. In gastric cancer, Fas expression is downregulated, and caspase-8-, caspase-3-, and poly (adenosine diphosphate-ribose) polymerase 1 (PARP1)-mediated cell apoptosis is altered.^{19,20} Autoimmune lymphoproliferative syndrome presents as an uncontrolled proliferation of lymphocytes caused by defective Fas.²¹ In A549 cells, neutrophils were found to arrest the tumor cell cycle via the Fas ligand/Fas pathway.²² In a bladder cancer study, Fas was found to participate in gallic acid-mediated bladder cancer cell proliferation and migration inhibition.²³ In association with Fas ligand, Fas promotes proliferation and migration of brain endothelial cells.²⁴ However, its role in cervical cancer remains poorly characterized.

In this study, we discovered the gene nuclear pore complex 210 (*NUP210*) to be overexpressed in cervical cancer. We investigated the expression of NUP210, miR-22, and Fas in cervical cancer tissues and their function in regulating HeLa cell activity. We also evaluated how NUP210 is regulated and how it regulates its downstream gene, *NUP210*.

MATERIALS AND METHODS

Clinical samples and ethical statement

At total of 33 paired clinical samples were collected from patients ranging in age from 40–60 years who did not have future fertility requirement and who underwent radical hysterectomy or pelvic lymphadenectomy in our hospital in 2018. All patients provided written informed consent. None of the patients received radiotherapy or chemotherapy before the sur-

gery. Pathological analysis revealed that all patients had squamous cell carcinoma, with clinical stages ranging from IB1 to IIA2 (FIGO 2009). Detailed patient information is presented in the Supplementary Table 2 (only online). Cancer tissues and adjacent normal tissue were paired and stored in a deep freezer (–80°C). All matters concerning clinical samples were reviewed and authorized by the Institutional Ethics Committee of The Third Affiliated Hospital of Soochow University [(2019)文第021号(快审)].

Immunohistochemistry

Cervical cancer tissues and paired adjacent normal tissues were processed for immunohistochemistry. All samples were formalin-fixed, paraffin-embedded, and immunostained with the NUP210 antibody (Abcam, Cambridge, MA, USA) and Fas antibody (Abcam). Quantification was conducted by counting positive cells per field. Data are presented as a mean±standard deviation (SD).

Cells and culture method

HeLa cells were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA) and preserved in our lab. HeLa cells were cultured as instructed by the ATCC in Eagle's Minimum Essential Medium (ATCC) supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) at 37°C under 5% CO₂.

293T cells used for lentivirus production were preserved in our lab and cultured in Dulbecco's Modified Eagle's Medium (Gibco) supplemented with 10% FBS at 37°C under 5% CO₂.

Establishment of the HeLa-sh-NUP210 cell line

The sh-NUP210 lentivirus expression vector was constructed by inserting NUP210 cDNA into the pLVX-IRES-ZsGREEN1 vector. Lentivirus was produced by transfecting the sh-NUP210 vector and the pSPAX2 and pMD2.G plasmids into 293T cells and collecting virus-containing supernatant at 72 h post transfection. The supernatant was added into HeLa cell culture medium and centrifuged at 132 g for 30 min. The sample was then incubated for another 24 h. Puromycin at 2 µg/mL was used to screen cells for 5 days.

miR-22 mimics and transfection

miR-22 mimics (5'-AAGCUGCCAGUUGAAGAACUGU-3') were synthesized by GenePharma (Shanghai, China). Diluted mimics and Lipofectamine 2000 (Thermo Fisher, Waltham, MA, USA) were mixed and incubated at room temperature for 10 min before being dispensed onto HeLa cells seeded a day before transfection.

Establishment of the miR-22-expressing cell line

The miR-22-expressing vector (miR-22 Ad) was constructed by inserting the miR-22 sequence into the pHBLV-CMV-IRES-Puro vector. Lentivirus was produced as described above, re-

placing the sh-NUP210 vector with miR-22 Ad. Infection of miR-22 lentivirus and screening for the stable cell pool were performed as described above.

Immunoblotting

Protein sample preparation

The cultured cells or clinical samples were lysed and ultrasonicated with sodium dodecyl sulfate lysis buffer (Beyotime, Shanghai, China) on ice for 5 min and denaturalized in a water bath at 100°C for 5 min. The supernatant was then transferred into new Eppendorf tubes and used to determine the resultant protein concentration using the Enhanced Bicinchoninic Acid Protein Assay Kit (Beyotime).

Western blotting

An equal amount of protein sample was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The blots were then incubated with NUP210 antibody (Abcam), Fas antibody (Abcam), Histone antibody (Abcam), or anti-GAPDH (MultiScience, Hangzhou, China) antibody at 4°C overnight, followed by incubation with HRP-conjugated goat anti-rabbit or goat anti-mouse antibody (MultiScience) for 1 hour at room temperature. The relative protein levels were detected using peroxide LumiGLO reagent (Cell Signaling Technology, Danvers, MA, USA) and quantified according to the ratio of gray value to the corresponding GAPDH or histone level. The experiments were repeated three times independently.

Reverse transcription followed by real-time quantitative PCR (RT-qPCR)

RNA was extracted using RNAiso Plus (TAKARA, Beijing, China) according to the manufacturer's instructions. The total RNA was then subjected to reverse transcription using a kit from GENEray (Shanghai, China). Finally, cDNA was used in the qPCR analysis. Relative RNA levels were normalized to that of the internal reference, and results are presented as a mean \pm SD. The primers used are presented in Supplementary Table 1 (only online), among which the miR-22 primers were chosen in reference to a published article.²⁵

Bioinformatic analysis of NUP210-binding miRNA and luciferase reporter assay

We used miRWalk (<http://129.206.7.150/>) to predict NUP210-binding miRNAs and found that miR-22 could bind to NUP210 CDS. The NUP210 CDS sequence was cloned into the pGL3 vector using the ClonExpress Ultra One Step Cloning kit (Vazyme, Nanjing, China). miR-22 mimics from GenePharma and pGL3-NUP210 were co-transfected into 293T cells using Lipofectamine 2000 (Thermo Fisher) according to the manufacturer's instructions. The luciferase activity was measured

using the Nano-Glo[®] Dual-Luciferase[®] kit from Promega (Madison, WI, USA). Each transfection experiment was performed in triplicate.

Cell apoptosis assay

The Annexin V-APC/7-AAD Apoptosis Detection kit (MultiScience) was used for cell staining and flow cytometry following the manufacturer's instructions. Briefly, the cells were washed twice with phosphate-buffered saline (PBS), and then 5×10^5 cells were collected and resuspended in 500 μ L of binding buffer. Thereafter, 5 μ L of Annexin V-APC was added and mixed well, and then 5 μ L of 7-AAD was added. The reaction was performed at room temperature (22–26°C) for 5 min in the dark, and Accuri C6 from BD Biosciences (Franklin Lake, NJ, USA) was used to detect apoptotic cells.

Cell cycle analysis

The Cell Cycle Staining Kit (MultiScience) was used for cell staining and flow cytometry following the manufacturer's instructions. Briefly, the cells were washed twice with PBS, and 5×10^5 cells were collected. The cells were resuspended in 1 mL of DNA staining solution. Then, 10 μ L of permeabilization solution was added and mixed well. The reaction was performed at room temperature for 30 min in the dark, and Accuri C6 from BD Biosciences was used to detect the cell cycle. Data were analyzed using FlowJo software (BD Biosciences).

Statistical analysis

The data are presented as a mean \pm SD. The comparison of means between two groups was conducted using Student's t-test. The results with $p < 0.05$ were considered significant (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

RESULTS

NUP210 is overexpressed in cervical cancer tissues at both the mRNA and protein level

We searched the NUP210 GEO Dataset (GSE9750) and found that NUP210 could be related to cancer development. We collected 33 pairs of clinical tissues (Supplementary Table 2, only online) from patients undergoing surgery in our hospital and analyzed NUP210 expression by immunohistochemistry. The results revealed higher NUP210 protein expression in cancer tissues (Fig. 1A). We then evaluated the expression of NUP210 mRNA in cancer tissues by RT-qPCR and the results showed comparatively higher levels of NUP210 mRNA expression in cervical cancer tissues (Fig. 1B). We randomly selected seven pairs of clinical samples and measured NUP210 protein level by western blotting and found that NUP210 was also overexpressed at the protein level (Fig. 1C).

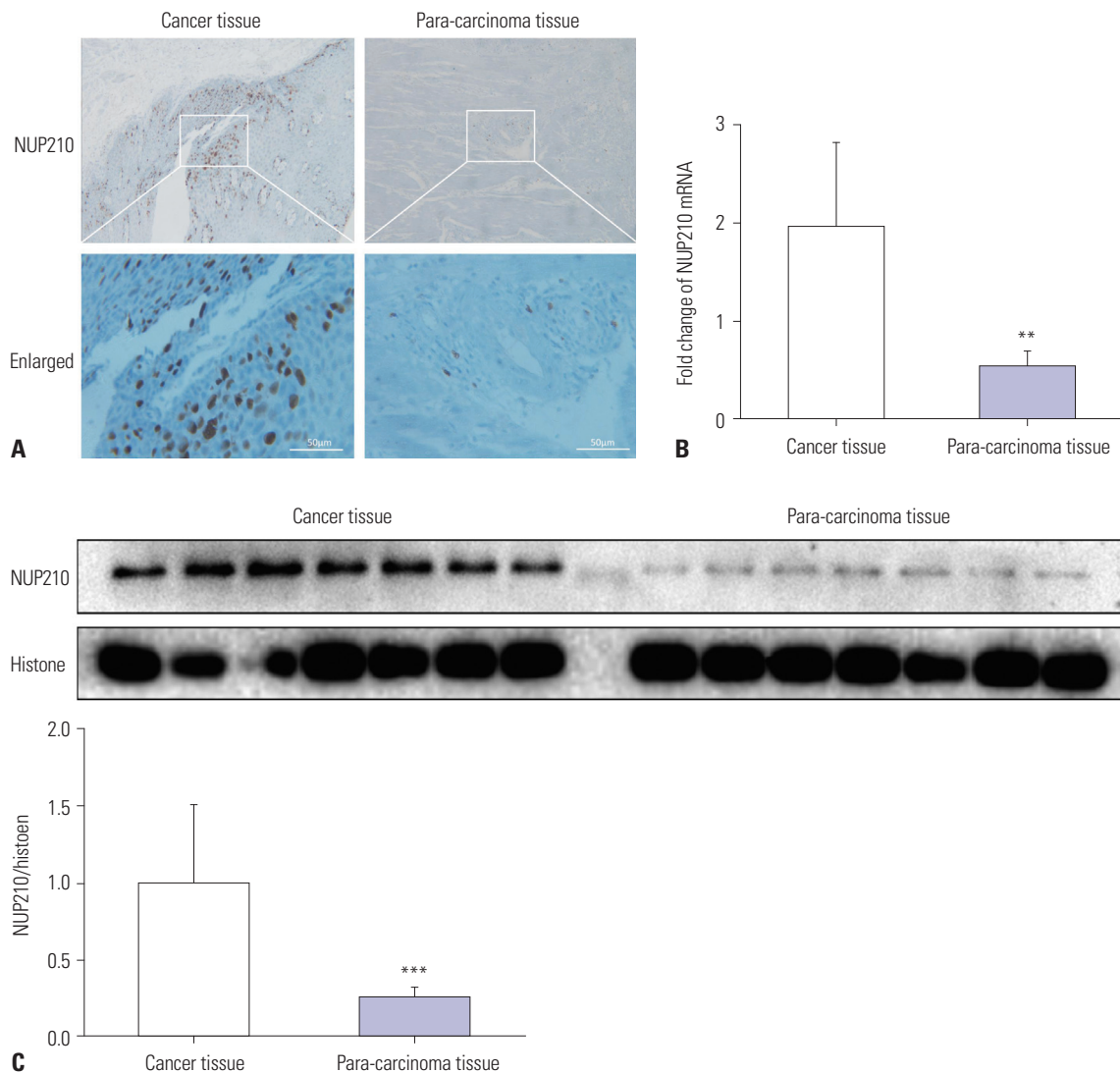


Fig. 1. NUP210 is overexpressed in cervical cancer tissues at both the mRNA and protein level. (A) NUP210 protein expression in cervical cancer tissue (left) and paired adjacent normal tissues (right) measured by immunohistochemistry. (B) *NUP210* mRNA expression in the 33 pairs of normal tissues and cancer tissues by RT-qPCR. (C) Western blotting of seven pairs of randomly selected clinical samples from the 33 pairs of samples (top) and quantification of NUP210 protein using normal tissues as the control. ** $p < 0.01$ and *** $p < 0.001$ for Student's t-test. RT-qPCR, real-time quantitative polymerase chain reaction.

Knockdown of NUP210 expression induces cell cycle arrest and apoptosis

To investigate the function of NUP210 in HeLa cells, we knocked down its expression by infecting HeLa cells with sh-NUP210 lentivirus (Fig. 2A). As measured by RT-qPCR (Fig. 2B) and Western blotting (Fig. 2C), NUP210 expression was efficiently knocked down. We then measured the apoptosis rate of both empty vector- and sh-NUP210-transformed cells. The data showed a considerably higher apoptosis rate in *NUP210* knock-down cells (Fig. 2D). We then evaluated the cell cycle and found that an increased number of sh-NUP210-transformed cells was accumulated in the G1 phase, indicating considerably lower cell proliferation, compared to that of control cells (Fig. 2E).

After knocking down NUP210 expression, we found that the HeLa cell cycle was arrested and that apoptosis was increased,

indicating that NUP210 overexpression in HeLa cells promote cell proliferation, contributing to tumor development.

miR-22 is downregulated in cervical cancer tissues and regulates the cell cycle

We used miRWalk to predict miRNAs binding to NUP210 and found that miR-22 might bind to NUP210 and regulate its expression. Data from GEO (GSE9750) also suggested miR-22 is inhibited in cervical cancer. This confirms data from one study that showed miR-22 targets alpha-enolase 1 to suppress retinoblastoma cell proliferation²⁶ and regulates human cervical squamous carcinoma cell proliferation.²⁷

We first measured miR-22 expression in the same clinical tissues used to evaluate NUP210 expression. The RT-qPCR results revealed lower miR-22 expression in cancer tissues (Fig.

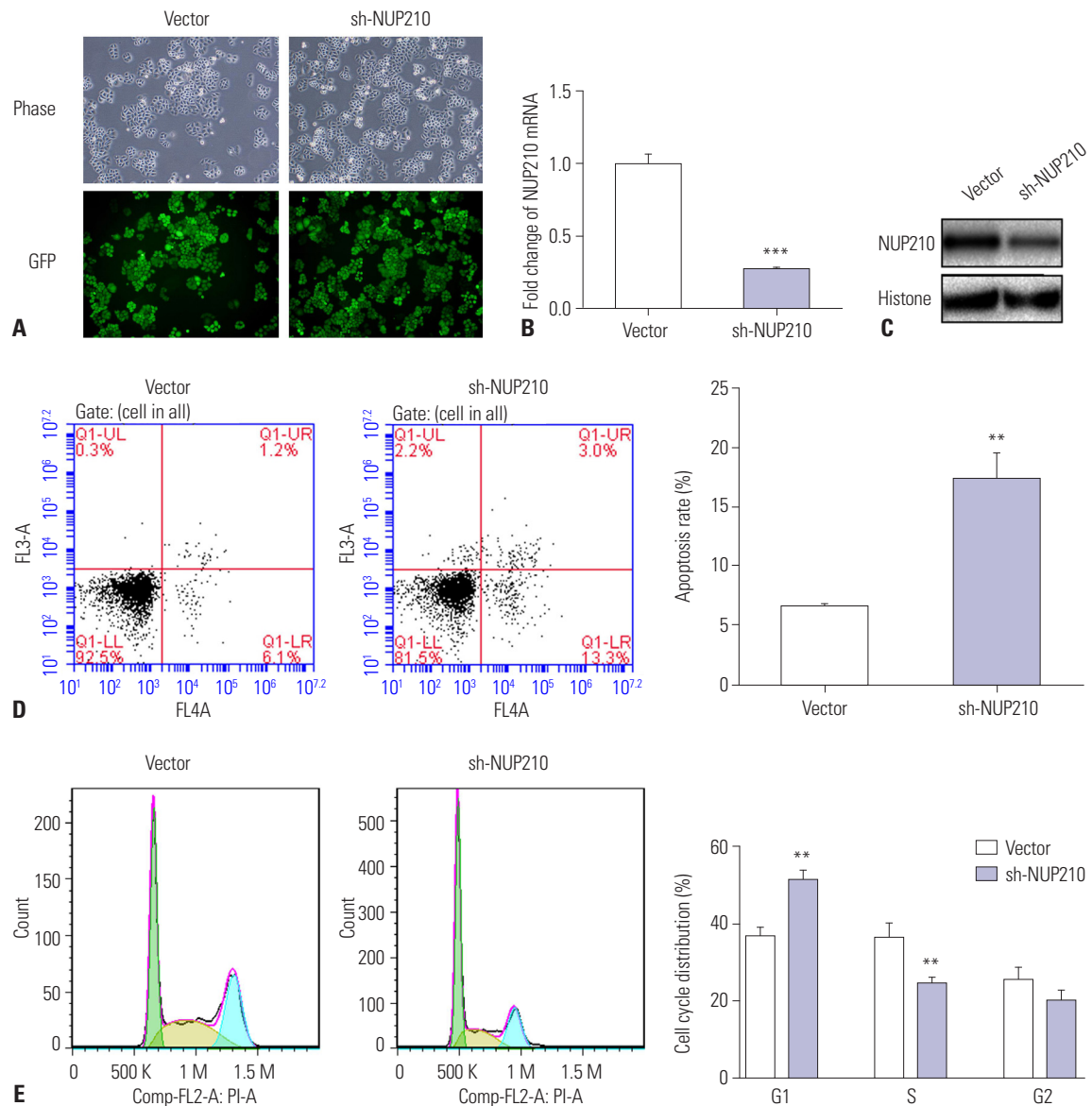


Fig. 2. Knockdown of NUP210 induces cell cycle arrest and apoptosis. (A) HeLa cells were transduced with sh-NUP210 lentiviruses (right) or its empty vector control (left). Representative images were taken under a light (top) and fluorescence microscope (bottom) ($\times 100$). (B) *NUP210* mRNA levels in treated cells as described in (A). (C) Western blotting of cells treated as described in (A) (top) and quantification of relative protein levels normalized to histone levels (bottom). (D) Apoptosis rate measured by flow cytometry (left) and percentage of apoptotic cells from three independent experiments (right). (E) Cell cycle analysis (left) by flow cytometry and quantification of cells distributed in different phases (right). ** $p < 0.01$ and *** $p < 0.001$ for Student's t-test.

3A). We then transfected HeLa cells with miR-22 mimics (Fig. 3B) and then measured NUP210 levels. The data showed that miR-22 overexpression inhibits NUP210 mRNA and protein expression (Fig. 3C and D).

To explore underlying mechanisms, we designed and cloned NUP210 CDS (WT and mutant) into the pGL3 vector (sequence presented in Fig. 3F). After transfecting HeLa cells with miR-22 mimics and NUP210 CDS vectors, we measured their luciferase activities. The results showed that miR-22 inhibited luciferase activity in the NUP210 CDS (WT) vector group (Fig. 3E).

Overall, the results revealed that miR-22 binds directly to

NUP210 CDS and inhibits its expression.

miR-22 overexpression induces cell cycle arrest and apoptosis

To elucidate the function of miR-22 in regulating HeLa cell activity, we transfected HeLa cells with miR-22 mimics (Fig. 4A). The RT-qPCR results showed that miR-22 was successfully expressed (Fig. 4B). Meanwhile, miR-22-expressing cells showed lower NUP210 expression at both the mRNA and protein level (Fig. 4C and 4D). The cells were then subjected to apoptosis assay and cell cycle analysis. The apoptosis assay showed an increased number of apoptotic miR-22-expressing cells (Fig.

4E); in addition, more cells were accumulated in the G1 phase, suggesting a lower cell proliferation rate from miR-22 overexpression (Fig. 4F).

miR-22-NUP210 signaling inhibits Fas expression to elicit cell cycle arrest and to promote cancer development

NUP210 is vital for CD4⁺ T lymphocyte survival in the periphery, and it functions by modulating STAT3-dependent Fas ex-

pression by regulating caveolin-2-mediated TCR signaling.¹⁷ To verify whether NUP210 regulates Fas expression in cervical cancer, we first determined the expression of Fas in cervical cancer tissues. We found considerably lower Fas expression at both the mRNA and protein level in cancer tissues, compared to that in paired normal tissues (Fig. 5A-C).

To investigate underlying mechanisms, Fas expression in NUP210-knockdown and miR-22 overexpressed HeLa cells was measured. HeLa cells transformed with sh-NUP210 or its

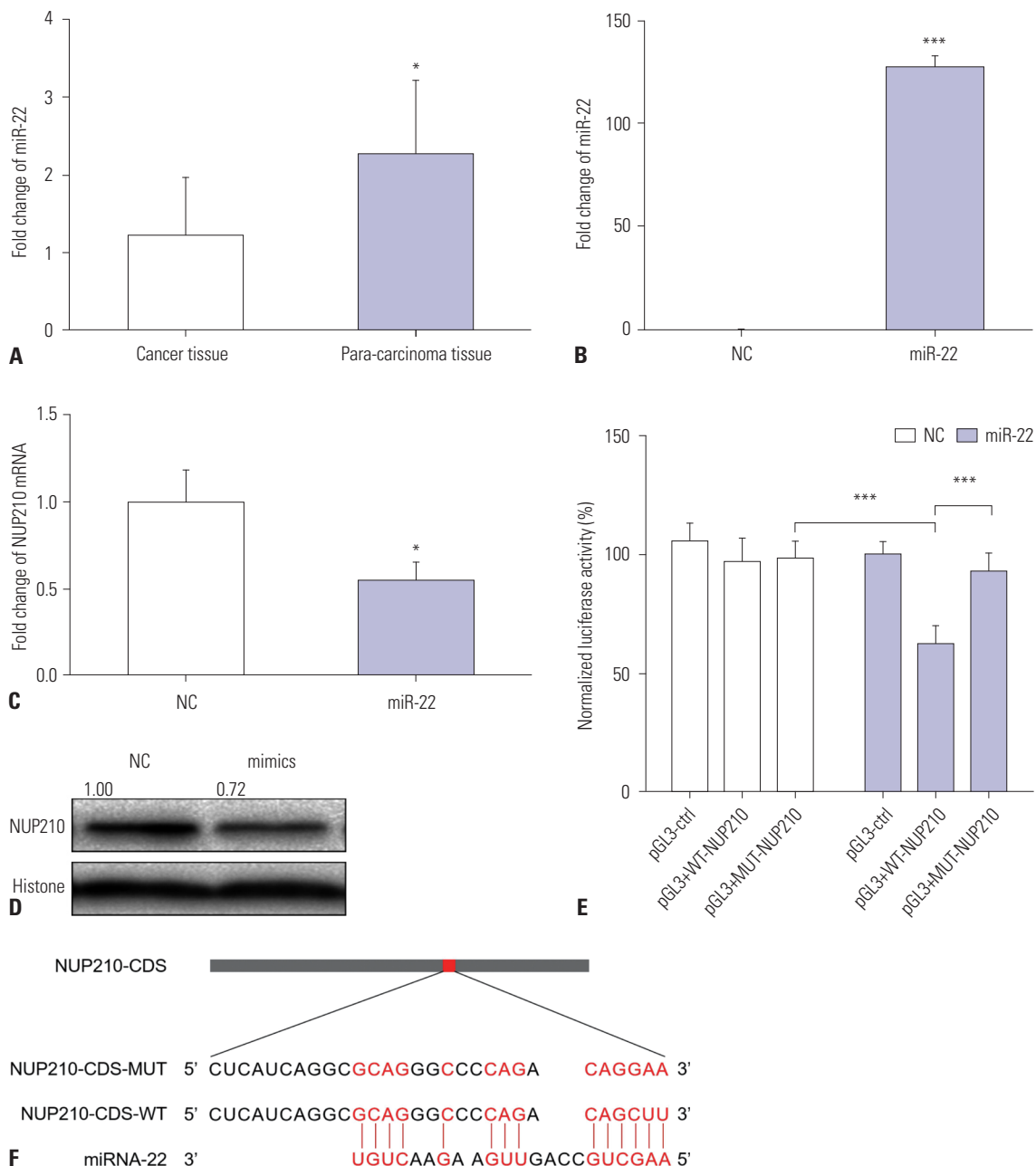


Fig. 3. miR-22 is downregulated in cervical cancer tissues and regulates the cell cycle. (A) Relative miR-22 levels in the 33 pairs of clinical samples described in Fig. 1A as normalized to U6 levels. (B) Relative miR-22 expression levels in HeLa cells transfected with miR-22 mimics or its negative control (NC). (C) *NUP210* mRNA levels in cells treated as described in (B) and normalized to histone levels. (D) Western blotting of cells treated as described in (B). (E) Luciferase reporter assay of 293T cells transfected with miR-22 mimics or its NC and pGL3-WT-NUP210, MUT-NUP210, or their vector. (F) Diagram of miR-22 binding to NUP210 CDS and mutant bases in mutant vector. * $p < 0.05$ and *** $p < 0.001$ for Student's t-test.

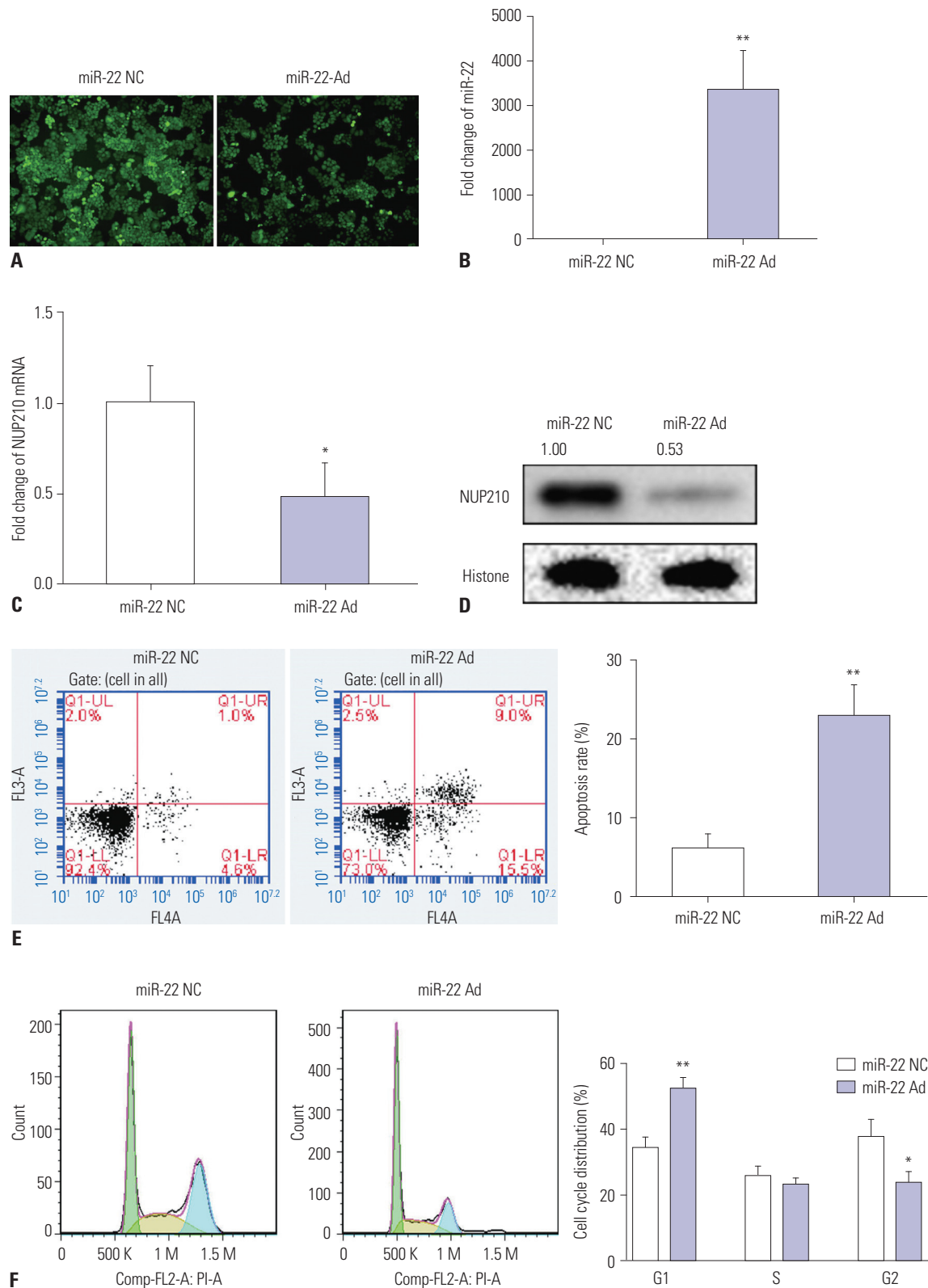


Fig. 4. miR-22 overexpression arrests the HeLa cell cycle and induces cell apoptosis. (A) HeLa cells were transfected with miR-22 mimics or its NC. Representative images were taken using a fluorescence microscope ($\times 100$). (B) miR-22 expression in cells treated as described in (A). (C) *NUP210* mRNA expression in cells treated as described in (A). (D) Western blotting of NUP210 expression in cells treated as described in (A) (top). (E) Apoptosis rate measured by flow cytometry (left) and percentage of apoptotic cells from three independent experiments (right). (F) Cell cycle analysis (left) by flow cytometry and quantification of cells distributed in different phases (right). * $p < 0.05$ and ** $p < 0.01$ for Student's t-test. NC, negative control.

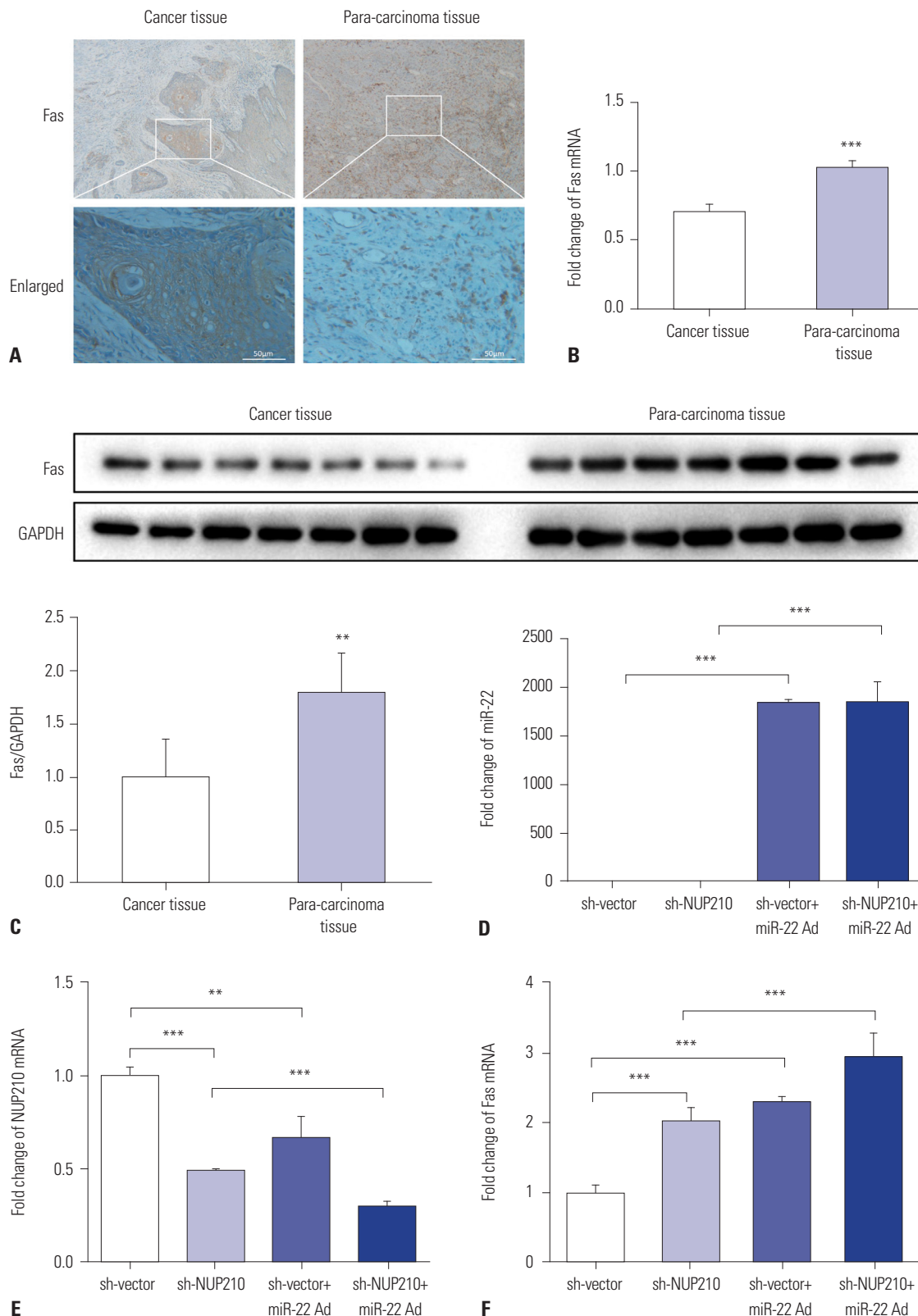


Fig. 5. miR-22-NUP210 signaling inhibits Fas expression to arrest the cell cycle and to promote cancer development. (A) Fas protein expression in cervical cancer tissue (left) and paired adjacent normal tissues (right) measured by immunohistochemistry. (B) Fas mRNA expression in the 33 pairs of normal tissues and cancer tissues by RT-qPCR. (C) Western blotting of seven pairs of randomly selected clinical samples from the 33 pairs of samples (top) and quantification of Fas protein using normal tissues as a control. (D) Relative miR-22 expression in sh-NUP210- or its empty vector-transformed HeLa cells transfected with miR-22 mimics or NC. (E) *NUP210* mRNA expression in cells treated as described in (D). (F) Fas mRNA expression in cells treated as described in (D). ** $p < 0.01$ and *** $p < 0.001$ for Student's t-test. RT-qPCR, real-time quantitative polymerase chain reaction; NC, negative control.

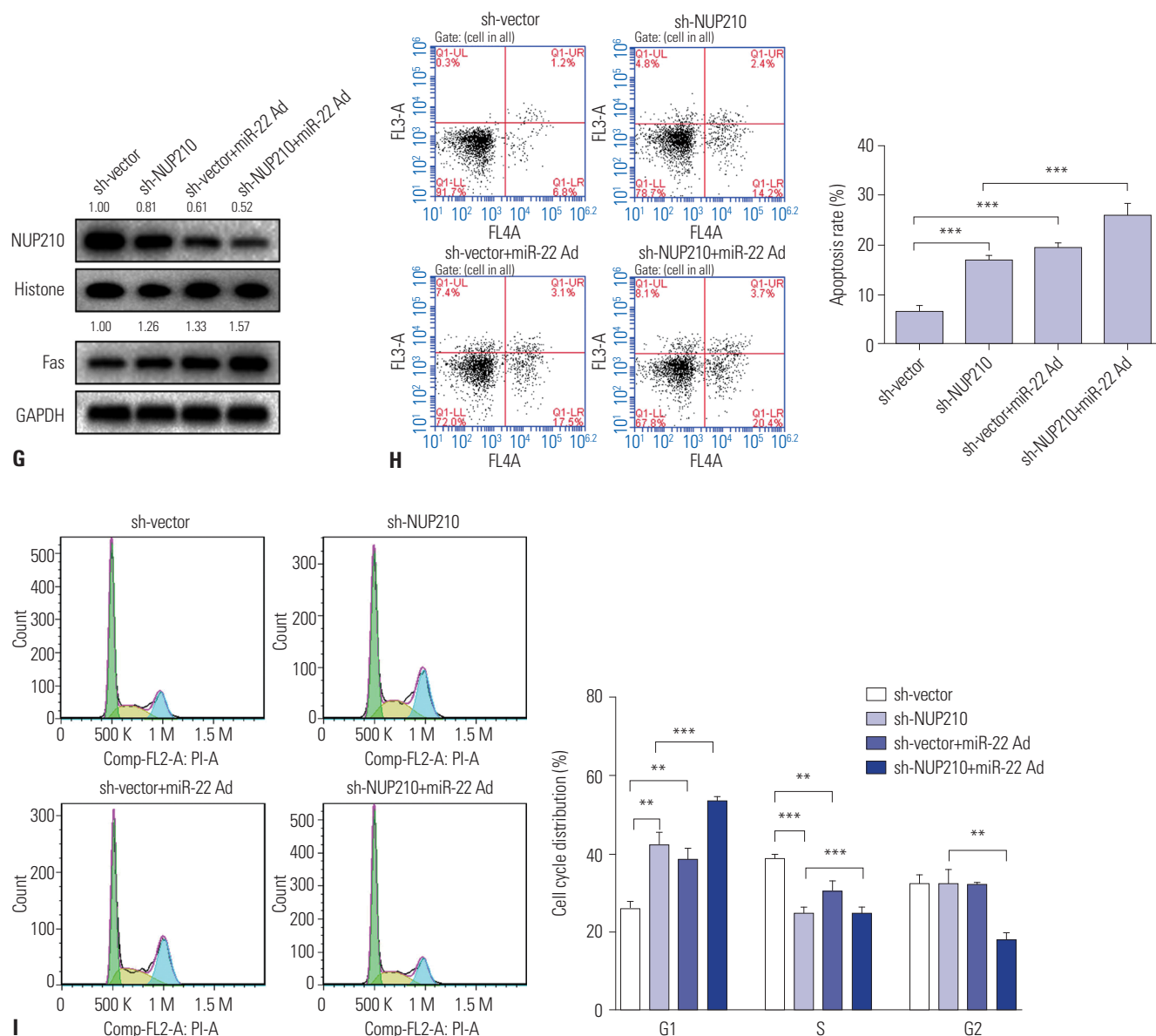


Fig. 5. miR-22-NUP210 signaling inhibits Fas expression to arrest the cell cycle and to promote cancer development. (G) Western blotting of Fas and NUP210 protein in cells treated as described in (D). (H) Apoptosis rate measured by flow cytometry (top) and percentage of apoptotic cells from three independent experiments (bottom). (I) Cell cycle analysis (left) by flow cytometry and quantification of cells distributed in different phases (right). ** $p < 0.01$ and *** $p < 0.001$ for Student's t-test.

vector lentiviruses were transfected with miR-22 mimics and its negative control (Fig. 5D) and then subjected to Western blotting. The results showed that after NUP210 knockdown (Fig. 5E and G) or miR-22 overexpression, Fas expression was promptly evaluated (Fig. 5F and G). These results suggest that Fas expression is regulated by the miR-22-NUP210 signal pathway.

Apoptosis and the cell cycle were analyzed to determine whether miR-22 overexpression and NUP210 knockdown, co-regulators of Fas overexpression, could modulate these mechanisms. Cell apoptosis analysis showed considerably more apoptotic cells after overexpressing miR-22 and knocking down NUP210 (Fig. 5H). Meanwhile, an increased number of cells was accumulated in the G1 phase, suggesting reduced proliferative

ability (Fig. 5I). Collectively, these results indicate that the miR-22-NUP210 signal pathway regulates cell apoptosis and arrests the cell cycle by regulating Fas expression.

DISCUSSION

In this study, we investigated the miR-22-NUP210-Fas axis in relation to cervical cancer cell activity. Our data revealed that NUP210 is overexpressed at the protein level in cervical cancer tissues and that its overexpression induces cell cycle arrest, leading to abnormal cell proliferation. By investigating NUP210-binding miRNAs, we found that miR-22 could di-

rectly bind to NUP210 CDS and alter its expression. Assessment of clinical samples revealed that miR-22 expression is lower than that in normal tissues. Using the luciferase reporter assay, we proved that miR-22 directly binds to NUP210 CDS and could inhibit its expression. In cervical cancer development, miR-22 expression was downregulated, resulting in NUP210 overexpression, leading to abnormal cell proliferation. We then found that Fas, which participates in cell cycle regulation, was regulated by the miR-22-NUP210 signal pathway. Overall, our study revealed that low miR-22 levels in cervical cancer induce higher NUP210 expression, causing Fas inhibition and cancer cell proliferation, leading to cancer development.

Although the function of NUP210 in cell differentiation has been identified, the expression and function of NUP210 in cancer development have not been well studied. In our study, we found that abnormal overexpression of NUP210 in cervical cancer promotes cancer cell proliferation. It is possible that this finding may translate to other cancer types, though additional studies analyzing NUP210 in tumorigenesis are warranted.

miR-22 is an important biomarker of several cancers. Research has been demonstrated that miR-22 targets Sp1 to inhibit hepatocellular carcinoma development.¹² In this study, we identified the direct binding of miR-22 and NUP210 CDS. Furthermore, we demonstrated that miR-22 inhibits NUP210 expression to regulate HeLa cell proliferation. In cervical cancer development, miR-22 expression was reduced, resulting in NUP210 overexpression and inducing cancer cell proliferation and cancer development.

Fas and its ligand, FasL, which belong to the tumor necrosis factor family, have been extensively studied. Fas-FasL binding is involved in the regulation of cell death in breast, bladder, and gastric cancers.^{18,19,23} We found the Fas expression was regulated by the miR-22-NUP210 regulation axis to facilitate cancer development. In the future, we aim to characterize the association between NUP210 and Fas, which will validate the findings of this study.

In conclusion, we comprehensively demonstrated that miR-22 inhibition in cervical cancer promotes NUP210 expression, in turn affecting the expression of Fas. Moreover, miR-22-NUP210 signaling alters Fas levels, contributing to cervical cancer development. This study provides insight into novel NUP210 functions other than differentiation and highlights one upstream regulator of miR-22. This suggests that NUP210 could serve as a novel target for cervical cancer diagnosis and treatment.

ACKNOWLEDGEMENTS

This study was supported in part by a grant from the Jiangsu Province Maternal and Child Health Research Projects (grant number F201869).

AUTHOR CONTRIBUTIONS

Conceptualization: Qiao Gu, Wenjie Hou, and Xiaoyuan Ni. **Data curation:** Qiao Gu and Wenjie Hou. **Formal analysis:** Qiao Gu, Wenjie Hou, and Xiaoyuan Ni. **Funding acquisition:** Xiaoyuan Ni. **Investigation:** Qiao Gu, Wenjie Hou, Huan Liu, Zonghao Zhu, Lijuan Shi, and Wenfeng Ye. **Methodology:** all authors. **Project administration:** Qiao Gu, Wenjie Hou, and Xiaoyuan Ni. **Resources:** Qiao Gu, Wenjie Hou, and Xiaoyuan Ni. **Software:** Qiao Gu and Huan Liu. **Supervision:** Qiao Gu, Wenjie Hou, and Xiaoyuan Ni. **Validation:** Qiao Gu, Wenjie Hou, Huan Liu, Zonghao Zhu, Lijuan Shi, and Wenfeng Ye. **Visualization:** Qiao Gu, Wenjie Hou, and Xiaoyuan Ni. **Writing—original draft:** Qiao Gu and Wenjie Hou. **Writing—review & editing:** Qiao Gu, Wenjie Hou, and Xiaoyuan Ni. **Approval of final manuscript:** all authors.

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REFERENCES

- Shafabakhsh R, Reiter RJ, Mirzaei H, Teymoordash SN, Asemi Z. Melatonin: a new inhibitor agent for cervical cancer treatment. *J Cell Physiol* 2019;234:21670-82.
- Gu XY, Zheng RS, Sun KX, Zhang SW, Zeng HM, Zou XN, et al. Incidence and mortality of cervical cancer in China, 2014. *Zhonghua Zhong Liu Za Zhi* 2018;40:241-6.
- Baloch Z, Yasmeen N, Li Y, Zhang W, Lu H, Wu X, et al. Knowledge and awareness of cervical cancer, human papillomavirus (HPV), and HPV vaccine among HPV-infected Chinese women. *Med Sci Monit* 2017;23:4269-77.
- Lin W, Wang Y, Liu Z, Chen B, Yuan S, Wu B, et al. Inequalities in awareness and attitude towards HPV and its vaccine between local and migrant residents who participated in cervical cancer screening in Shenzhen, China. *Cancer Res Treat* 2020;52:207-17.
- Lin W, Wang Y, Liu Z, Chen B, Yuan S, Wu B, et al. Awareness and attitude towards human papillomavirus and its vaccine among females with and without daughter(s) who participated in cervical cancer screening in Shenzhen, China. *Trop Med Int Health* 2019;24:1054-63.
- Wen Y, Pan XF, Zhao ZM, Chen F, Fu CJ, Li SQ, et al. Knowledge of human papillomavirus (HPV) infection, cervical cancer, and HPV vaccine and its correlates among medical students in Southwest China: a multi-center cross-sectional survey. *Asian Pac J Cancer Prev* 2014;15:5773-9.
- Gomez-Cavazos JS, Hetzer MW. The nucleoporin gp210/Nup210 controls muscle differentiation by regulating nuclear envelope/ER homeostasis. *J Cell Biol* 2015;208:671-81.
- D'Angelo MA, Gomez-Cavazos JS, Mei A, Lackner DH, Hetzer MW. A change in nuclear pore complex composition regulates cell differentiation. *Dev Cell* 2012;22:446-58.
- Hussein NA, Kholy ZA, Anwar MM, Ahmad MA, Ahmad SM. Plasma miR-22-3p, miR-642b-3p and miR-885-5p as diagnostic biomarkers for pancreatic cancer. *J Cancer Res Clin Oncol* 2017; 143:83-93.

10. Ma J, Shang S, Wang J, Zhang T, Nie F, Song X, et al. Identification of miR-22-3p, miR-92a-3p, and miR-137 in peripheral blood as biomarker for schizophrenia. *Psychiatry Res* 2018;265:70-6.
11. van Boven N, Akkerhuis KM, Anroedh SS, Rizopoulos D, Pinto Y, Battes LC, et al. Serially measured circulating miR-22-3p is a biomarker for adverse clinical outcome in patients with chronic heart failure: the Bio-SHiFT study. *Int J Cardiol* 2017;235:124-32.
12. Chen J, Wu FX, Luo HL, Liu JJ, Luo T, Bai T, et al. Berberine upregulates miR-22-3p to suppress hepatocellular carcinoma cell proliferation by targeting Sp1. *Am J Transl Res* 2016;8:4932-41.
13. Jiang W, Han X, Wang J, Wang L, Xu Z, Wei Q, et al. miR-22 enhances the radiosensitivity of small-cell lung cancer by targeting the WRNIP1. *J Cell Biochem* 2019;120:17650-61.
14. Nahand JS, Taghizadeh-Boroujeni S, Karimzadeh M, Borran S, Pourhanifeh MH, Moghoofei M, et al. microRNAs: new prognostic, diagnostic, and therapeutic biomarkers in cervical cancer. *J Cell Physiol* 2019;234:17064-99.
15. Sadri Nahand J, Moghoofei M, Salmaninejad A, Bahmanpour Z, Karimzadeh M, Nasiri M, et al. Pathogenic role of exosomes and microRNAs in HPV-mediated inflammation and cervical cancer: a review. *Int J Cancer* 2020;146:305-20.
16. Ghasemi F, Shafiee M, Banikazemi Z, Pourhanifeh MH, Khanbabaee H, Shamshirian A, et al. Curcumin inhibits NF- κ B and Wnt/ β -catenin pathways in cervical cancer cells. *Pathol Res Pract* 2019;215:152556.
17. Borlido J, Sakuma S, Raices M, Carrette F, Tinoco R, Bradley LM, et al. Nuclear pore complex-mediated modulation of TCR signaling is required for naïve CD4⁺ T cell homeostasis. *Nat Immunol* 2018;19:594-605.
18. Bębenek M, Duś D, Koźlak J. Prognostic value of the Fas/Fas ligand system in breast cancer. *Contemp Oncol (Pozn)* 2013;17:120-2.
19. Wang X, Fu Z, Chen Y, Liu L. Fas expression is downregulated in gastric cancer. *Mol Med Rep* 2017;15:627-34.
20. Mottolese M, Buglioni S, Bracalenti C, Cardarelli MA, Ciabocco L, Giannarelli D, et al. Prognostic relevance of altered Fas (CD95)-system in human breast cancer. *Int J Cancer* 2000;89:127-32.
21. Rieux-Laucat F, Magérus-Chatinet A, Neven B. The autoimmune lymphoproliferative syndrome with defective FAS or FAS-ligand functions. *J Clin Immunol* 2018;38:558-68.
22. Sun B, Qin W, Song M, Liu L, Yu Y, Qi X, et al. Neutrophil suppresses tumor cell proliferation via Fas/Fas ligand pathway mediated cell cycle arrested. *Int J Biol Sci* 2018;14:2103-13.
23. Liao CC, Chen SC, Huang HP, Wang CJ. Gallic acid inhibits bladder cancer cell proliferation and migration via regulating fatty acid synthase (FAS). *J Food Drug Anal* 2018;26:620-7.
24. Zhang C, Gao F, Teng F, Zhang M. Fas/FasL complex promotes proliferation and migration of brain endothelial cells via FADD-FLIP-TRAF-NF- κ B pathway. *Cell Biochem Biophys* 2015;71:1319-23.
25. Shen J, Zhang J, Jiang X, Wang H, Pan G. LncRNA HOX transcript antisense RNA accelerated kidney injury induced by urine-derived sepsis through the miR-22/high mobility group box 1 pathway. *Life Sci* 2018;210:185-91.
26. Liu Y, Li H, Liu Y, Zhu Z. MiR-22-3p targeting alpha-enolase 1 regulates the proliferation of retinoblastoma cells. *Biomed Pharmacother* 2018;105:805-12.
27. Lv KT, Liu Z, Feng J, Zhao W, Hao T, Ding WY, et al. MiR-22-3p regulates cell proliferation and inhibits cell apoptosis through targeting the eIF4EBP3 gene in human cervical squamous carcinoma cells. *Int J Med Sci* 2018;15:142-52.