Clinics in Oncology

Research Article
Published: 28 Jan, 2025

Microrna-4429 Negatively Affects Colorectal Cancer Cell Invasion and Migration *via* Regulating SMAD3-Induced Epithelial-Mesenchymal Transition

*Zhe Zhang[1], Yongxi Wang[2], Zhuo Chen[2], Guozheng Xu[2], Tingting Zhao[2] and Fang Wang[2]**

[1]*Department of Oncology, The First Affiliated Hospital of Zhengzhou University, China*

[2]*Department of Breast Surgery, The First Affiliated Hospital of Zhengzhou University, China*

Abstract

Colorectal Cancer (CRC) is one common carcinoma and the fourth primary cause of cancer-caused death, with dismal prognosis. Studies have reported that miR-4429 develops anti-cancer function in follicular thyroid carcinoma and non-small cell lung cancer. However, its regulation mechanism in CRC and whether miR-4429 is implicated in the regulation of pathological activities are not investigated. The aim of our study was to explore the potent role of miR-4429 in CRC. In the present study, miR-4429 was a low-expressed gene in CRC cells and gain-of-function experiments displayed that miR-4429 promotion inhibited cell proliferation, migration, invasion and EMT process. It was viewed from mechanism assays that miR-4429 targeted Forkhead box M1 (FOXM1) and therefore regulate SMAD family member 3 (SMAD3) expression. Rescue experiments exposed that miR-4429 influenced cell proliferation, migration, invasion and EMT process in CRC by modulation of FOXM1-activated SMAD3. Our study detected that miR-4429 targeted FOXM1 to decrease SMAD3 expression and impeded cell proliferation, migration and invasion and EMT process of CRC cells, offering better direction in respect of investigation of the mechanisms in CRC and promising therapeutic targets for CRC.

Keywords: Colorectal cancer (CRC); miR-4429; FOXM1; SMAD3

OPEN ACCESS

Correspondence:
Fang Wang, Department of Breast Surgery, The First Affiliated Hospital of Zhengzhou University, No.1 Jianshe East Road, Zhengzhou, 450052, Henan, China,
E-mail: w13019510625@163.com

Received Date: *16 Jan 2025*
Accepted Date*: 27 Jan 2025*
Published Date*: 28 Jan 2025*

Citation:
Zhang Z, Wang Y, Chen Z, Xu G, Zhao T, Wang F. Microrna-4429 Negatively Affects Colorectal Cancer Cell Invasion and Migration via Regulating SMAD3-Induced Epithelial-Mesenchymal Transition. Clin Oncol. 2025; 10: 2122.
ISSN: 2474-1663

Copyright *© 2025 Fang Wang. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Introduction

On the basis of global cancer statistics, Colorectal Cancer (CRC) is the third most usual carcinoma and the fourth primary cause of cancer-caused death [1-3]. The 5-year survival rate radically reduces from 90% (at the early stage) to 10% (at the late stage) [4]. Hence, the early diagnosis of CRC by identifying more regulatory molecules is an imperative task.

In the human genome, only 1 to 2% genes encodes proteins and the other genes are non-coding RNAs (ncRNAs) involving small ncRNAs (microRNAs, piRNAs and siRNAs) with the length of 18-25 nucleotides and long ncRNAs (lncRNAs) with at least 200 nucleotides in length [5-8]. Increasing evidence has reported that aberrantly expressed microRNAs (miRNAs) are associated with the progression of CRC, either play oncogenic roles or develop tumor suppressor functions in the biological processes [5,9,10]. For examples, miR-622 works as a tumor suppressor and targets E2F1 directly in esophageal squamous cell carcinoma [11]; miR-27a boosts EMT process in ovarian cancer by activating Wnt/β-catenin signalling *via* targeting FOXO1 [12]; miR-548, transcriptionally reduced by HIF1α/HDAC1, represses tumorigenesis of pancreatic cancer by decreasing Vimentin expression [13]. However, the contribution of miRNAs to CRC is still not completely researched.

MicroRNA 4429 (miR-4429) has been demonstrated to inhibit the progression of human tumors. Long noncoding RNA LINC00313 mediates papillary thyroid cancer initiation by sponging miR-4429 [14]. MiR-4429 represses tumor progression and Epithelial-Mesenchymal Transition (EMT) via absorbing CDK6 in clear cell renal cell carcinoma [15]. MiR-4429 sensitized cervical cancer cells to radiation via targeting RAD51 [16]. Moreover, the expression pattern and function role of miR-4429 in CRC has never been explored.

In the present study, we identified that miR-4429 was downregulated in CRC and miR-4429 promotion inhibited cell proliferation, migration and invasion, as well as repressed EMT process. Furthermore, miR-4429 was proved to weaken SMAD3 by targeting FOXM1, a transcription factor of SMAD3. Rescue experiments affirmed that miR-4429 modulated the cellular activities of CRC

via SMAD3. The aim of this research is to investigate the role of miR-4429 in the biological processes of CRC, especially in cell motility.

Materials and Methods

Cell lines and cell culture

The Cell Bank of Typical Culture Collection (Chinese Academy of Sciences, Shanghai, China) provided the needed cells comprising normal human colon epithelial cells (FHC) and CRC cell lines (SW620, LoVo, HT29 and HCT116). And Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) with 100U/ml penicillin, 0.1mg/ml streptomycin and 10% fetal bovine serum (FBS, Gibco, USA) was utilized to culture these cells in a humid incubator with 5% CO_2 at 37°C.

Cell transfection

For overexpression of FOXM1 or SMAD3, FOXM1 or SMAD3 coding sequence was synthesized and subcloned into pcDNA3.1 vectors (pcDNA3.1/FOXM1 or pcDNA3.1/SMAD3), with empty pcDNA3.1 vectors as negative control. For knockdown of FOXM1 or SMAD3, FOXM1 or SMAD3-specific shRNAs were established, compared with negative control shNC. To overexpress miR-4429, miR-4429 mimics was established, compared to NC mimics. All vectors were gained from Genepharma (Shanghai, China). In line with the supplier's protocols, transfections were conducted with Lipofectamine 2000 (Invitrogen).

Quantitative real-time PCR (qPCR)

Total RNA was harvested from HCT116 and HT29 cells by TRIzol Reagent (Invitrogen) following the directed instructions. The synthesis of cDNA was carried out by employing High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem) with random hexamer primers. qPCR was performed utilizing SYBR Green reagent (Applied Biosystem). Gene expression was calculated with the use of the comparative cycle threshold (CT) (2-ΔCT and 2-ΔΔCT) method. U6 served as an internal control for miR-4429 and Glyceraldehyde 3-Phosphate-Dehydrogenase (GAPDH) served as an endogenous control for other 8 genes.

Microarray analysis

Under the transfection of NC mimics or miR-4429 mimics, differentially expressed mRNAs in tumor cells were scanned through microarray analysis and the heatmap was plotted. The top ten down-regulated genes were respectively TFAP2B, FOXM1, SEMA3A, ZNF652, MEX3B, SMAD3, AK4, HOXB4, HNRNPC and KDM5A.

Cell proliferation assays

The cell viability of CRC cells was evaluated through Cell Counting Kit-8 (CCK-8) assay referring to the manufacturer's guidelines. Briefly, 1×103 cells were planted into 96-pore dishes at indicated point-in-times and subsequently supplemented with 10 μl CCK-8 solution. Absorbance at the wavelength of 450 nm was determined via a microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA).

In 5-Ethynyl-2′-Deoxyuridine (EdU) assay, cells at exponential phase were inoculated into 6-well culture dishes with indicated doses of EDU reagent for nearly 3 h. Following washing twice with Phosphate Buffered Saline (PBS) for transient 5 min, cells were cultured with 4% paraformaldehyde for half an hour. Next, samples were permeated utilizing 0.3% TritonX-100 in PBS and stained with reaction solution (C0075S, Beyotime, China) before washing twice by PBS for 5 min. The images were obtained via Nikon microscopy with 20× and 40× visions.

As for colony formation assay, cells at a density of 50 cells per plate were seeded into plates and grown for two weeks. The culture media were discarded and the cells were washed using PBS for two times. Cells were firstly fixed with 4% paraformaldehyde for a quarter and stained in Giemsa solution for about 20 minutes, followed by washing with water and air drying. A mesh transparent film was used to overlay the inverted dishes. Clones larger than 50 cells were counted under a microscope.

Cell migration and invasion assay

For examination of cell migration, transfected CRC cells were seeded onto the upper chamber with medium containing 1% FBS, and the lower chamber was loaded with full growth medium. 1 d later, the cells were rinsed and fastened in 4% paraformaldehyde for about 0.5 h. Next, cells in the upper layer were removed with caution by a cotton swab. Crystal violet was adopted to stain the membranes for nearly 30 min. Cell counting in each well was manipulated on five fields randomly. For cell invasion, a similar process was performed, except for the addition of the Matrigel-pre-coated membrane.

Immunofluorescence (IF)

CRC cells were plated on coverslips that were placed in the bottom of each pore in 12 culture dishes. 48 h after transfection, following fasten by 4% PFA solution at RT for half an hour, cells were washed using washing buffer. Non-specific binding was hindered with block solution including 10% FBS. Cells were grown in anti-E-cadherin or anti-N-cadherin-prepared block solution (Santa Cruz) for 30 min at RT, then washed twice by washing buffer, incubated for 30 min in secondary Alexa fluor 488 donkey anti-mouse IgG (A-21202, Thermo Fisher). Mounting solution was adopted to mount coverslips on slides and LSM 800 (Zeiss, Jena, Germany) inverted confocal microscope equipped with a 63x Plan-Apochromat objective (NA1.4 oil) was utilized for capturing all images.

Luciferase reporter assay

For miR-4429 and FOXM1 luciferase reporter assay, the FOXM1 sequences possessing Wild-Type (WT) or Mutant (MUT) miR-4429 binding sites were separately synthesized and then cloned into pmirGLO luciferase vectors (GeneCreat, China), forming FOXM1-WT or FOXM1-MUT reporter vectors. MiR-4429 mimics or NC mimics was co-transfected with FOXM1-WT or FOXM1-MUT into CRC cells. After 48 h of transfection, cells were extracted and the luciferase activities were analyzed utilizing the dual-luciferase reporter assay system (Promega). As for FOXM1 and SMAD3 promoter luciferase reporter assay, the SMAD3 promoter was constructed in to pmirGLO luciferase vectors and then co-transfected with shNC or shFOXM1 into tumor cells. The effect of miR-4429 on SMAD3 promoter was also performed via transfection of SMAD3 promoter plasmids and NC mimics or miR-4429 mimics.

RNA pull-down assay

In brief, CRC cells were transfected with the 3'end biotinylated miR-4429-WT (sense) or miR-4429-Mut (antisense) for 1d, compared to biotinylated-NC probes, reaching a final concentration of 20 nmol/L. Subsequently, cells were co-cultivated with streptavidin-coated magnetic beads (Ambion, Life Technologies). qPCR was performed to analyze the abundance of FOXM1 in bound fractions after the pull-down of biotin-coupled RNA complex.

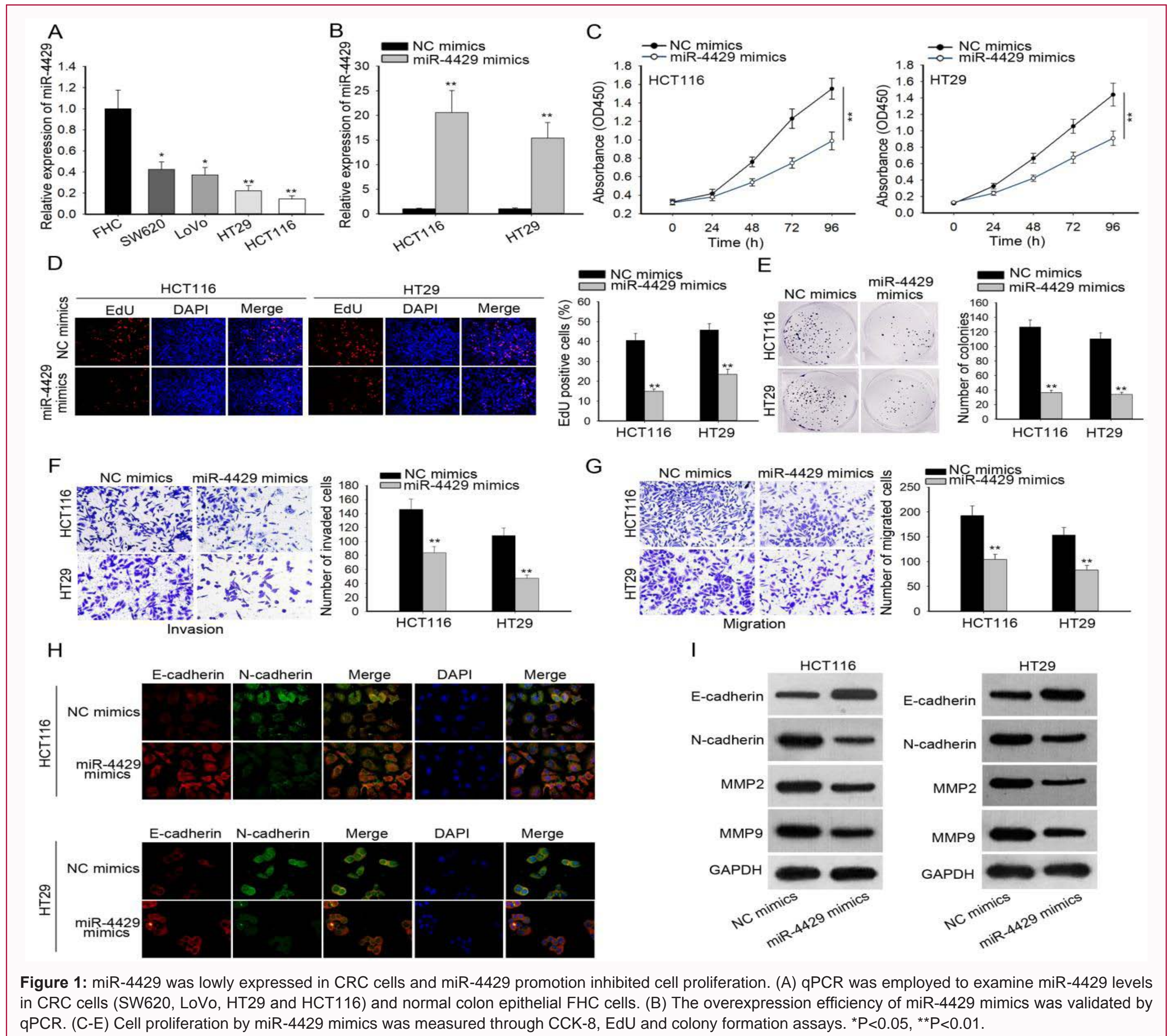

Figure 1: miR-4429 was lowly expressed in CRC cells and miR-4429 promotion inhibited cell proliferation. (A) qPCR was employed to examine miR-4429 levels in CRC cells (SW620, LoVo, HT29 and HCT116) and normal colon epithelial FHC cells. (B) The overexpression efficiency of miR-4429 mimics was validated by qPCR. (C-E) Cell proliferation by miR-4429 mimics was measured through CCK-8, EdU and colony formation assays. *P<0.05, **P<0.01.

Chromosome immunoprecipitation (ChIP) assay

ChIP assay was implemented to testify the interplay between FOXM1 and SMAD3 promoter via using the ChIP assay kit (Beyotime, China). In short, fixed cells by 1% formaldehyde solution for 20 min were subsequently quenched with 0.125M glycine for approximately 10 min. DNA was sonicated into fragments from 200 to 300 bp. For each immunoprecipitation, antibodies against FOXM1 and IgG (Abcam) were used. Finally, the immunoprecipitated DNA was determined *via* qPCR.

Western blot

Post transfection, proteins were isolated by RIPA buffer along with phosphatase inhibitor cocktail and proteinase inhibitor cocktail. After being separated through 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), proteins were transferred to Polyvinylidene Fluoride (PVDF) membranes. The membranes were cultivated with primary antibodies all night at 4 following the block with BSA for 2 h, followed by culturing with secondary antibody for extra 2 h. After that, the membranes were washed thrice using tris-buffered saline/Tween 20, grown with Super Signal West Pico chemiluminescent substrate and further dissected with the help of the GeneGnome HR Image Capture System. The primary antibodies were antibodies against TFAP2B, FOXM1, SEMA3A, ZNF652, MEX3B, SMAD3, AK4, HOXB4, HNRNPC, KDM5A; E-cadherin, N-cadherin, MMP2, MMP9 and GAPDH (all from Abcam).

Statistical analysis

Statistical analysis from separate and triplicated assays was manipulated using SPSS version 17.0 and the data are denoted as mean ± SD. The differences between groups were evaluated by Adopting One-Way Analysis Of Variance (ANOVA) and the student's t-test. Statistics with a p value below 0.05 was considered as significant.

Research data for this article

The data generated in this study are available within the article and its supplementary data files (Supplementary Figure 1-3).

Results

MiR-4429 promotion inhibited cell proliferation

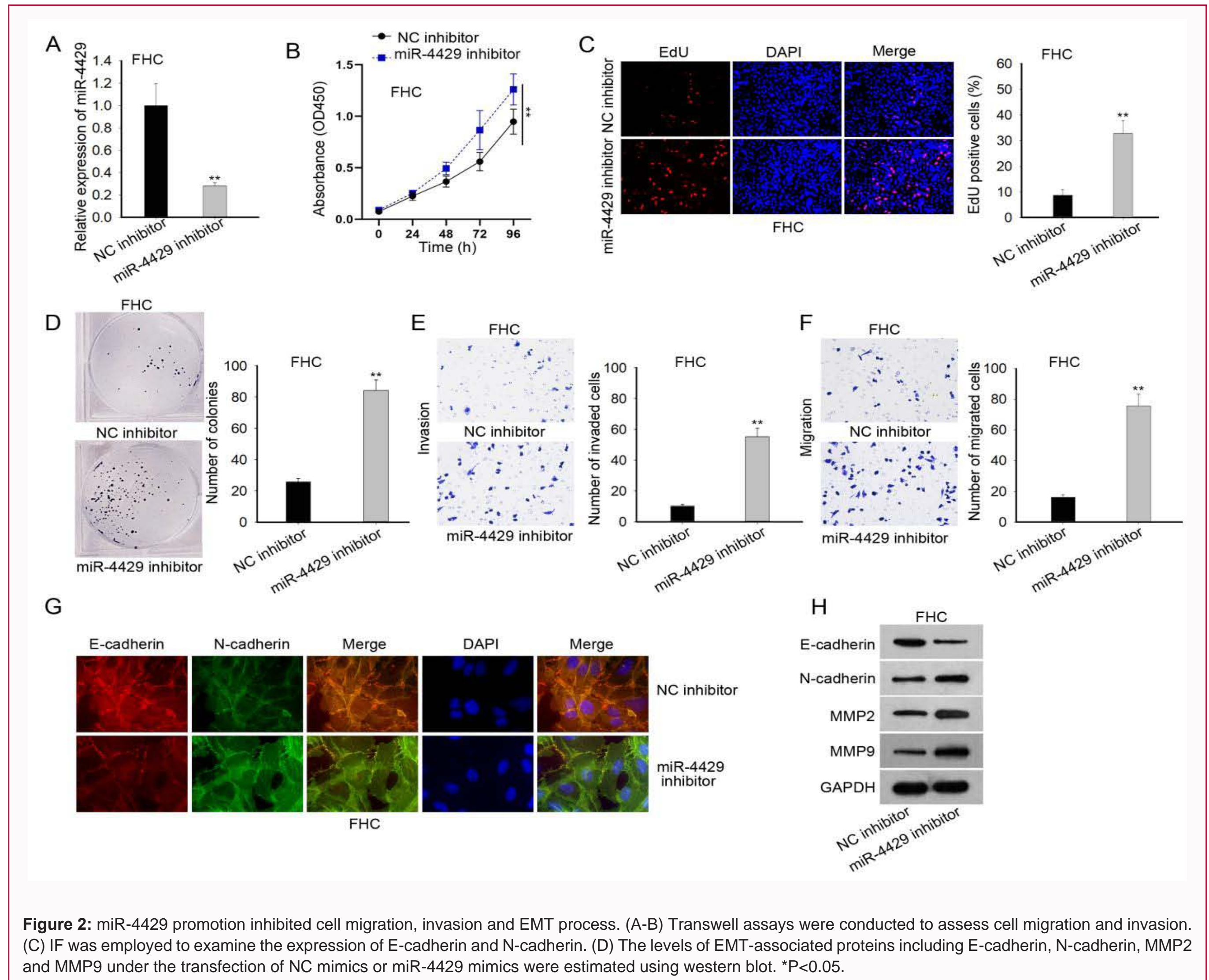

Figure 2: miR-4429 promotion inhibited cell migration, invasion and EMT process. (A-B) Transwell assays were conducted to assess cell migration and invasion. (C) IF was employed to examine the expression of E-cadherin and N-cadherin. (D) The levels of EMT-associated proteins including E-cadherin, N-cadherin, MMP2 and MMP9 under the transfection of NC mimics or miR-4429 mimics were estimated using western blot. *P<0.05.

In order to probe the function of miR-4429 in Colorectal Cancer (CRC), we initially examined the expression level of miR-4429 in CRC cells comprising SW620, LoVo, HT29 and HCT16 cells and normal colon epithelial FHC cells. The results showed that miR-4429 was expressed at low levels in CRC cells, especially in HT29 and HCT16 cells (Figure 1A). Then miR-4429 expression in HCT116 and HT29 cells was upregulated by treatment of miR-4429 mimics for gain-of-function assays (Figure 1B). In CCK-8 assay, cell viability was overtly restrained by miR-4429 up-regulation (Figure 1C). In EdU assay and colony formation assay, cell proliferation was significantly repressed when miR-4429 was overexpressed (Figure 1D,E). These experiments elucidated that the overexpression of miR-4429 suppressed cell proliferation.

MiR-4429 promotion also repressed cell migration, invasion and EMT process

Subsequently, we continued to inquire the effects of miR-4429 on cell motility of CRC cells. Through Transwell assays, we observed that cell migration and invasion were both controlled after the transfection of miR-4429 mimics in HCT116 and HT29 cells (Figure 2A,B). IF analysis indicated that E-cadherin levels were increased while N-cadherin levels were decreased after miR-4429 was elevated in HCT116 and HT29 cells (Figure 2C). As for EMT process, we measured the levels of Epithelial-Mesenchymal Transition (EMT)-associated proteins and found that E-cadherin levels were increased but the levels of N-cadherin, MMP2 and MMP9 were decreased by miR-429 mimics, which hinted that EMT process was suppressed by miR-4429 promotion (Figure 2D). These data told us that miR-4429 promotion restrained cell motility in CRC.

MiR-4429 might affect SMAD3 expression *via* FOXM1

MicroRNAs are widely known to affect cellular activities through working on the degradation of target mRNAs [17]. The heatmap dissected 200 differential genes in face of the transfection of miR-4429 mimics (Figure 3A). In HCT116 and HT29 cells, we estimated the mRNA and protein levels of ten most silenced mRNAs by miR-4429 mimics and disclosed that FOXM1 and SMAD3 levels were dramatically downregulated (Figure 3B,C). The results of qPCR and western blotting indicated that FOXM1 or SMAD3 levels were highly expressed in CRC cells, compared with FHC cells (Figure 3D,E). What's more, FOXM1 could modulate SMAD3 expression whereas SMAD3 could not regulated FOXM1 expression in turn (Figure 3F-I). Subsequently, luciferase reporter assay determined that miR-4429 up-regulation obviously impaired the luciferase activity of SMAD3

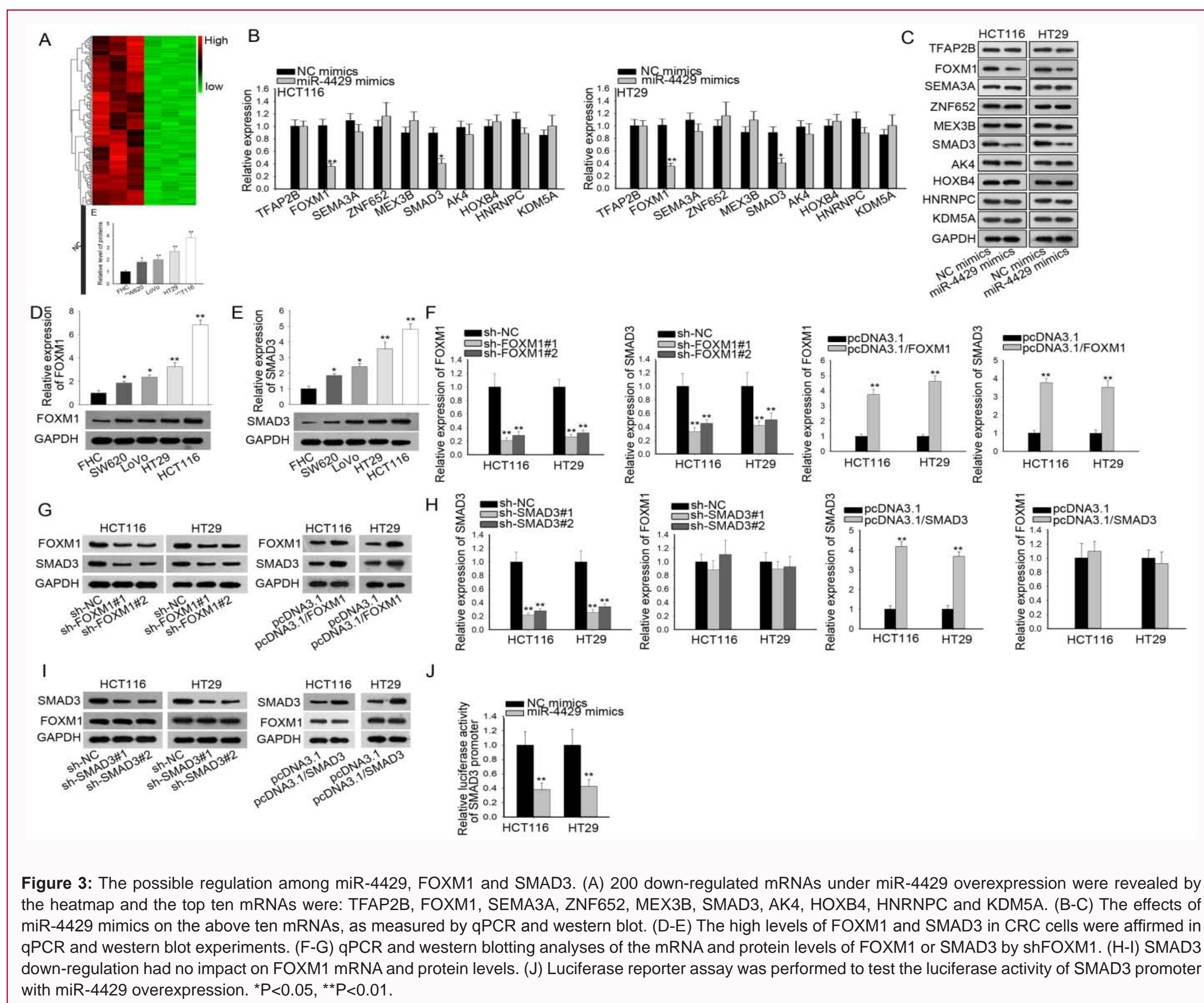

Figure 3: The possible regulation among miR-4429, FOXM1 and SMAD3. (A) 200 down-regulated mRNAs under miR-4429 overexpression were revealed by the heatmap and the top ten mRNAs were: TFAP2B, FOXM1, SEMA3A, ZNF652, MEX3B, SMAD3, AK4, HOXB4, HNRNPC and KDM5A. (B-C) The effects of miR-4429 mimics on the above ten mRNAs, as measured by qPCR and western blot. (D-E) The high levels of FOXM1 and SMAD3 in CRC cells were affirmed in qPCR and western blot experiments. (F-G) qPCR and western blotting analyses of the mRNA and protein levels of FOXM1 or SMAD3 by shFOXM1. (H-I) SMAD3 down-regulation had no impact on FOXM1 mRNA and protein levels. (J) Luciferase reporter assay was performed to test the luciferase activity of SMAD3 promoter with miR-4429 overexpression. *P<0.05, **P<0.01.

promoter (Figure 3J). Through searching prediction websites, we noted that FOXM1 was one target of miR-4429 but SMAD3 was not, and we surprisingly found out the assumed transcriptional regulation of FOXM1 on SMAD3. Taken these data into speculate that there are some regulation mechanisms among miR-4429, FOXM1 and SMAD3. Totally, SMAD3 expression might be reduced by miR-4429 due to its inhibition on FOXM1.

MiR-4429 targeted FOXM1 to regulate SMAD3

Sequentially, we attempted to verify the mechanism by which miR-4429 regulated SMAD3 expression transcriptionally. For luciferase reporter assays, we mutated the binding sites of FOXM1 for miR-4429. The wild and mutant types of FOXM1 sequences and the binding sequences of miR-4429 were shown in Figure 4A. The results demonstrated that only the luciferase activity of FOXM1-WT was hindered by miR-4429 mimics (Figure 4B). RNA pull-down experiment confirmed the interaction between miR-4429 and FOXM1 (Figure 4C). Next, ChIP and luciferase reporter assays validated the interaction between FOXM1 and SMAD3 promoter (Figure 4D,E). To affirm the co-impacts of miR-4429 and FOXM1 on SMAD3, two cells were infected with NC mimics, miR-4429 mimics, miR-4429 mimics + pcDNA3.1/FOXM1. qPCR and western blotting testified the transfection efficacy (Figure 4F). ChIP assays were conducted again and the results showed that the binding of FOXM1 and SMAD3 promoter was impeded by promotion of miR-4429 but restored by upregulation of FOXM1 (Figure 4G). And SMAD3 mRNA and protein levels were decreased when miR-4429 expression was augmented, and this phenomenon was reversed when FOXM1 expression was fortified (Figure 4H,I). In summary, miR-4429 modulated SMAD3 expression by targeting FOXM1.

Upregulation of SMAD3 reversed the inhibitory effects of miR-4429 overexpression on cellular activities of CRC cells

The whole regulation mechanism of miR-4429 in CRC was confirmed by rescue experiments. The overexpression efficiency of pcDNA3.1/SMAD3 was determined by western blot (Figure 5A). As observed by CCK-8, EdU and colony formation assays, miR-4429 mimics hampered cell proliferation whereas this effect was partly abrogated by co-transfection of pcDNA3.1/SMAD3 (Figure 5B-D). In transwell assays, cell migration and invasion numbers were bated by increase of miR-4429, which were recovered in part by promotion of SMAD3 (Figure 5E,F). Moreover, the miR-4429 overexpression-inhibited EMT process was sort of facilitated through SMAD3

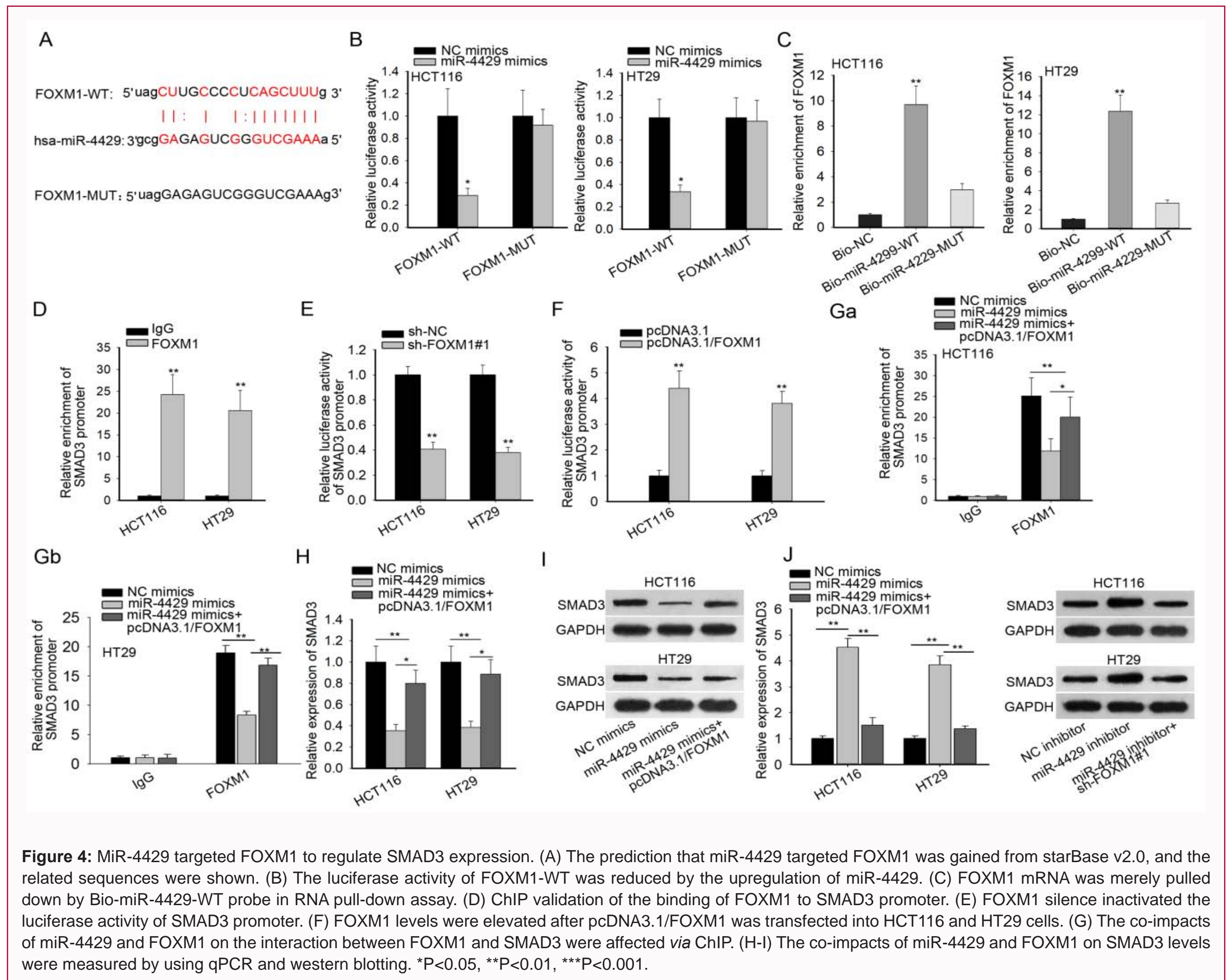

Figure 4: MiR-4429 targeted FOXM1 to regulate SMAD3 expression. (A) The prediction that miR-4429 targeted FOXM1 was gained from starBase v2.0, and the related sequences were shown. (B) The luciferase activity of FOXM1-WT was reduced by the upregulation of miR-4429. (C) FOXM1 mRNA was merely pulled down by Bio-miR-4429-WT probe in RNA pull-down assay. (D) ChIP validation of the binding of FOXM1 to SMAD3 promoter. (E) FOXM1 silence inactivated the luciferase activity of SMAD3 promoter. (F) FOXM1 levels were elevated after pcDNA3.1/FOXM1 was transfected into HCT116 and HT29 cells. (G) The co-impacts of miR-4429 and FOXM1 on the interaction between FOXM1 and SMAD3 were affected *via* ChIP. (H-I) The co-impacts of miR-4429 and FOXM1 on SMAD3 levels were measured by using qPCR and western blotting. *P<0.05, **P<0.01, ***P<0.001.

promotion based on that the enlargement of E-cadherin protein levels and the decline of N-cadherin, MMP2 and MMP9 protein levels was changed reversely (Figure 5G,H). In conclusion, miR-4429 modulated the proliferation and the motility *via* SMAD3 in CRC.

Discussion

Since Colorectal Cancer (CRC) is the third most common malignancy in the globe [3], many investigators have focused on the characterization of CRC-related microRNAs (miRNAs) as new biomarkers for diagnosis or targeted therapy [18-20]. In this study, miR-4429, a tumor repressor gene in cancer [14-16], was probed. Gain-of-function assays, for the first time, indicated that miR-4429 overexpression suppressed cell proliferation, migration and invasion, as well as restrained Epithelial-Mesenchymal Transition (EMT) process in CRC.

In the heatmap of decreased genes by miR-4429 mimics, only forkhead box M1 (FOXM1) and SMAD family member 3 (SMAD3) showed obvious decline under transfection of miR-4429 mimics in HCT16 and HT29 cells. In previous reports, FOXM1 has been demonstrated to be an oncogene and make great contribution to the transcription of genes so as to affect the biological activities of carcinomas [21-23]. And the tumorigenic role of SMAD3 was also revealed by numerous reports [24-27]. In subsequence, the monodirectional modulation of FOXM1 on SMAD3 was excavated. Strikingly, the repression of miR-4429 on SMAD3 promoter was experimented. The presumed targeting of FOXM1 by miR-4429 was unveiled but the regulation between miR-4429 and SMAD3 was not clear. Also, we obtained the predicted transcription factor role of FOXM1 for SMAD3. Transcription factors are generally known as regulators of genes at transcriptional level [28-30]. For instances, STAT3 facilitates AGC kinases activity in melanoma by transactivation of PDK1 [31]; SP1-induced LINC00511 enhances glioma progression by targeting miR-124-3p/CCND2 axis [32]; c-MYC allows transcription and splicing of the carcinogenic splicing factor Sam68 in cancer [33]. Thereafter, our researchers continued to attest the modulatory mechanism underlying miR-4429 in CRC. Mechanism assays displayed that miR-4429 targeted FOXM1 to lower SMAD3 expression. The correlation among the three genes was firstly elucidated.

In the end, rescue assays certified that upregulation of SMAD3 neutralized the inhibitory effects of miR-4429 overexpression on proliferative, migratory and invasive abilities as well as EMT process in CRC. Totally, our study indicated that miR-4429 targeted FOXM1 to decrease SMAD3 expression and hindered CRC cell proliferation,

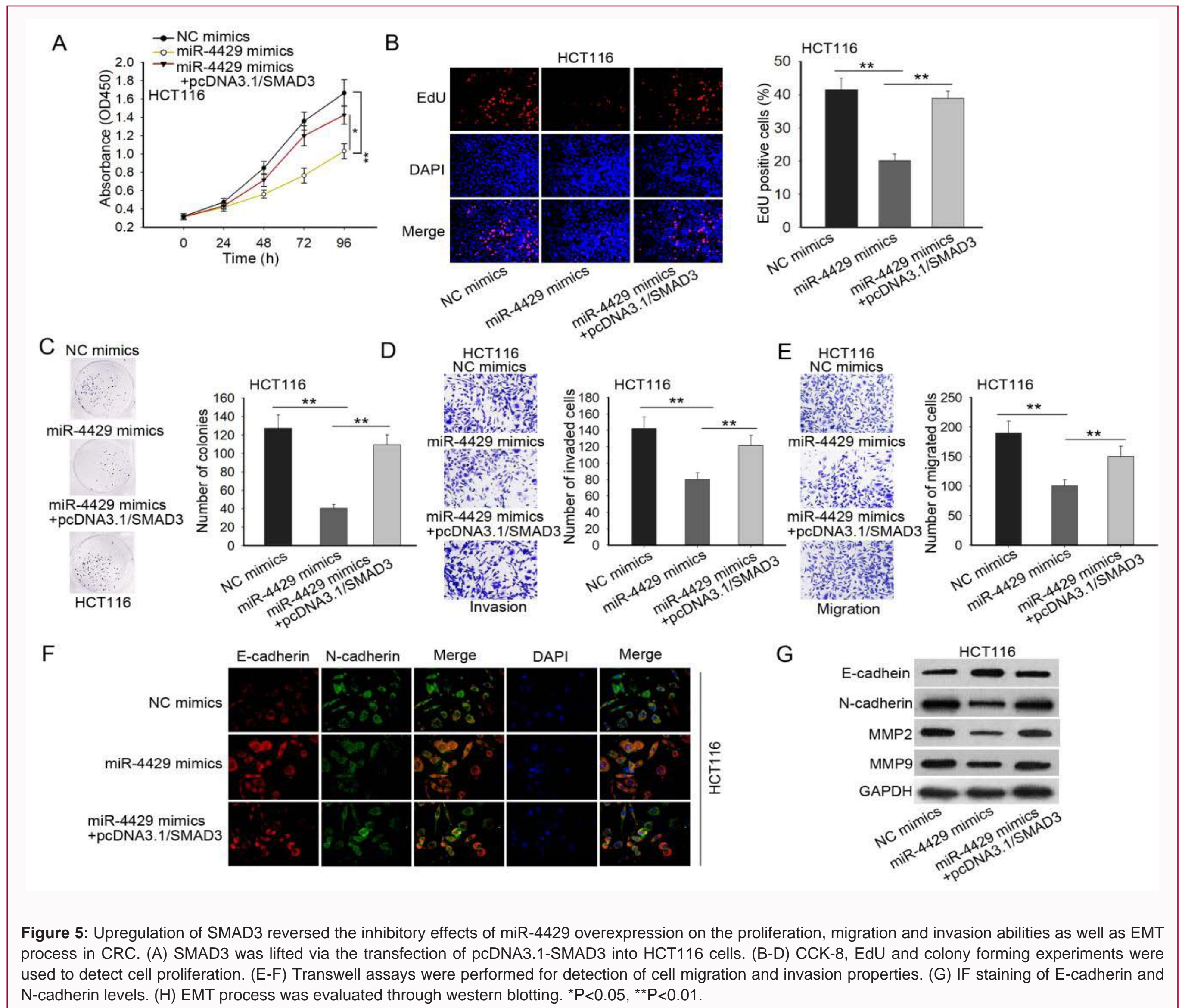

Figure 5: Upregulation of SMAD3 reversed the inhibitory effects of miR-4429 overexpression on the proliferation, migration and invasion abilities as well as EMT process in CRC. (A) SMAD3 was lifted via the transfection of pcDNA3.1-SMAD3 into HCT116 cells. (B-D) CCK-8, EdU and colony forming experiments were used to detect cell proliferation. (E-F) Transwell assays were performed for detection of cell migration and invasion properties. (G) IF staining of E-cadherin and N-cadherin levels. (H) EMT process was evaluated through western blotting. *P<0.05, **P<0.01.

migration and invasion and EMT process, which provided better direction in respect of investigation of the mechanisms in CRC.

Funding

This study was supported by Joint construction project of medical science and technology research plan of Henan Province (2018020095).

References

1. Brody H. Colorectal cancer. Nature. 2015;521(7551):S1.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-86.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87-108.
4. Moghimi-Dehkordi B, Safaee A. An overview of colorectal cancer survival rates and prognosis in Asia. World J Gastrointest Oncol. 2012;4(4):71-5.
5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-97.
6. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. Nature. 2012;489(7414):101-8.
7. Jia H, Osak M, Bogu GK, Stanton LW, Johnson R, Lipovich L. Genome-wide computational identification and manual annotation of human long noncoding RNA genes. RNA. 2010;16(8):1478-87.
8. Mattick JS, Makunin IV. Non-coding RNA. Hum Mol Genet. 2006;15(1):R17-29.
9. Jiang L, Wang R, Fang L, Ge X, Chen L, Zhou M, et al. HCP5 is a SMAD3-responsive long non-coding RNA that promotes lung adenocarcinoma metastasis via miR-203/SNAI axis. Theranostics. 2019;9(9):2460-74.
10. Yang ZG, Ma XD, He ZH, Guo YX. miR-483-5p promotes prostate cancer cell proliferation and invasion by targeting RBM5. Int Braz J Urol. 2017;43(6):1060-7.
11. Song C, Lu P, Shi W, Sun G, Wang G, Huang X, et al. MiR-622 functions as a tumor suppressor and directly targets E2F1 in human esophageal squamous cell carcinoma. Biomed Pharmacother. 2016;83:843-9.
12. Griesing S, Kajino T, Tai MC, Liu Z, Nakatochi M, Shimada Y, et al. Thyroid transcription factor-1-regulated microRNA-532-5p targets KRAS and MKL2 oncogenes and induces apoptosis in lung adenocarcinoma

cells. Cancer Sci. 2017;108(7):1394-1404.

13. Zhu S, He C, Deng S, Li X, Cui S, Zeng Z, et al. MiR-548an, Transcriptionally Downregulated by HIF1alpha/HDAC1, Suppresses Tumorigenesis of Pancreatic Cancer by Targeting Vimentin Expression. Mol Cancer Ther. 2016;15(9):2209-19.

14. Wu WJ, Yin H, Hu JJ, Wei XZ. Long noncoding RNA LINC00313 modulates papillary thyroid cancer tumorigenesis via sponging miR-4429. Neoplasma. 2018;65(6):933-42.

15. Pan H, Hong Y, Yu B, Li L, Zhang X. miR-4429 Inhibits Tumor Progression and Epithelial-Mesenchymal Transition Via Targeting CDK6 in Clear Cell Renal Cell Carcinoma. Cancer Biother Radiopharm. 2019;34(5):334-41.

16. Sun H, Fan G, Deng C, Wu L. miR-4429 sensitized cervical cancer cells to irradiation by targeting RAD51. J Cell Physiol. 2020;235(1):185-93.

17. Uddin A, Chakraborty S. Role of miRNAs in lung cancer. J Cell Physiol. 2018.

18. Huang L, Zhang Y, Li Z, Zhao X, Xi Z, Chen H, et al. MiR-4319 suppresses colorectal cancer progression by targeting ABTB1. United European Gastroenterol J. 2019;7(4):517-28.

19. Liu Y, Zhang Y, Wu H, Li Y, Zhang Y, Liu M, et al. miR-10a suppresses colorectal cancer metastasis by modulating the epithelial-to-mesenchymal transition and anoikis. Cell Death Dis. 2017;8(4):e2739.

20. Ng L, Wan TM, Man JH, Chow AK, Iyer D, Chen G, et al. Identification of serum miR-139-3p as a non-invasive biomarker for colorectal cancer. Oncotarget. 2017;8(16):27393-400.

21. Gartel AL. FOXM1 in Cancer: Interactions and Vulnerabilities. Cancer Res. 2017;77(12):3135-9.

22. Mittermair E, Krenn L, Marian B. Prenylated xanthones from metaxya rostrata suppress FoxM1 and induce active cell death by distinct mechanisms. Phytomedicine. 2019;60:152912.

23. Zhang J, Niu Y, Huang C. Role of FoxM1 in the Progression and Epithelial to Mesenchymal Transition of Gastrointestinal Cancer. Recent Pat Anticancer Drug Discov. 2017;12(3):247-59.

24. Jiang M, Shi L, Yang C, Ge Y, Lin L, Fan H, et al. miR-1254 inhibits cell proliferation, migration, and invasion by down-regulating Smurf1 in gastric cancer. Cell Death Dis. 2019;10(1):32.

25. Liu X, Wu Y, Zhou Z, Huang M, Deng W, Wang Y, et al. Celecoxib inhibits the epithelial-to-mesenchymal transition in bladder cancer via the miRNA-145/TGFBR2/Smad3 axis. Int J Mol Med. 2019;44(2):683-93.

26. Sakai S, Ohhata T, Kitagawa K, Uchida C, Aoshima T, Niida H, et al. Long Noncoding RNA ELIT-1 Acts as a Smad3 Cofactor to Facilitate TGFbeta/Smad Signaling and Promote Epithelial-Mesenchymal Transition. Cancer Res. 2019;79(11):2821-38.

27. Wang Y, Xiang J, Wang J, Ji Y. Downregulation of TGF-β1 suppressed proliferation and increased chemosensitivity of ovarian cancer cells by promoting BRCA1/Smad3 signaling. Biol Res. 2018;51(1):58.

28. Jiang Y, Wang D, Ren H, Shi Y, Gao Y. Oncogenic HBXIP enhances ZEB1 through Sp1 to accelerate breast cancer growth. Thorac Cancer. 2018;9(12):1664-70.

29. Joo YJ, Ficarro SB, Soares LM, Chun Y, Marto JA, Buratowski S. Downstream promoter interactions of TFIID TAFs facilitate transcription reinitiation. Genes Dev. 2017;31(21):2162-74.

30. Warfield L, Ramachandran S, Baptista T, Devys D, Tora L, Hahn S. Transcription of Nearly All Yeast RNA Polymerase II-Transcribed Genes Is Dependent on Transcription Factor TFIID. Mol Cell. 2017;68(1):118-29.e5.

31. Picco ME, Castro MV, Quezada MJ, Barbero G, Villanueva MB, Fernández NB, et al. STAT3 enhances the constitutive activity of AGC kinases in melanoma by transactivating PDK1. Cell Biosci. 2019;9:3.

32. Li C, Liu H, Yang J, Yang J, Yang L, Wang Y, et al. Long noncoding RNA LINC00511 induced by SP1 accelerates the glioma progression through targeting miR-124-3p/CCND2 axis. J Cell Mol Med. 2019;23(6):4386-94.

33. Caggiano C, Pieraccioli M, Panzeri V, Sette C, Bielli P. c-MYC empowers transcription and productive splicing of the oncogenic splicing factor Sam68 in cancer. Nucleic Acids Res. 2019;47(12):6160-71.