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Original Research Article

MiR-143-5p inhibits proliferation, invasion, and epithelial to mesenchymal transition of colorectal cancer cells by downregulation of HMGA2

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Abstract

Purpose: *To investigate the regulatory effect and molecular mechanism of miR-143-5p in colorectal cancer (CRC) progression.*
Methods: *Expression of miR-143-5p in CRC cell lines SW620 and HCT116 was determined by quantitative real-time polymerase chain reaction (qRT-PCR). Stable miR-143-5p overexpression was mediated by lentivirus. The effects of miR-143-5p on proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) of SW620 and HCT116 cells were assessed by colony formation assay, CCK-8, Transwell assay, wound healing assay, and western blot. Target prediction was performed for miR-143-5p, and a dual luciferase assay was used to verify the targeting relationship.*
Results: *Compared to CRC cells transfected with negative controls, cell proliferation, migration and invasion, and EMT were inhibited in miR-143-5p-overexpressing cells. Expression of HMGA2 (high-mobility Group AT-Hook 2), a target gene of miR-143-5p, was repressed by miR-143-5p. Rescue experiments confirmed that upregulation of HMGA2 due to mIR-143-5p overexpression reversed inhibition of CRC cell proliferation, invasion and EMT.*
Conclusion: *MiR-143-5p inhibits the malignant progression of CRC by regulating HMGA2 expression and is expected to provide new therapeutic approaches for clinical treatment of CRC.*

Keywords: *MiR-143-5p, High-mobility Group AT-Hook 2, HMGA2, Colorectal cancer, Epithelial-mesenchymal transition, EMT, Cell proliferation*

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INTRODUCTION

According to Global Cancer Statistics 2020 [1], colorectal cancer (CRC) is one of the top three cancers in terms of the percentage of new cases. Although the availability of colorectal microscopy has effectively reduced the incidence of this highly metastatic disease, there is still a need to investigate the underlying molecular mechanisms of CRC progression to improve patient prognosis. MicroRNAs (miRNAs) are generally evolutionarily conserved and regulate specific

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biological functions by binding RNA to cause silencing of genes [2,3]. In organisms, chromosomal rearrangements, epigenetic alterations, and point mutations lead to miRNA expression dysregulation in cancer [4,5]. This phenomenon is correlated with the pathogenesis of CRC. For example, upregulation of miR-27a is associated with impaired mitochondrial activity and oxidative phosphorylation in CRC cells [6]. Studies have shown that miR-1285-3p and miR-450a-5p exert oncogenic or antitumor effects, respectively, in CRC by regulating the expression of their target genes. Mining the molecules that affect the progression of CRC is beneficial in the development of clinical treatment strategies (pmid:32244500, 32144236).

MiR-143-5p, one of the most frequently dysregulated circulating miRNAs in CRC, is a prognostic marker candidate miRNA for CRC [7,8]. However, a lack of studies on the effect of miR-143-5p in CRC progression has prevented assessment of whether miR-143-5p may serve as a CRC therapeutic target. This study was conducted to investigate whether miR-143-5p acts as a cancer suppressor in CRC progression and to elucidate the molecular mechanisms involved in miR-143-5p regulation of CRC growth and metastasis.

EXPERIMENTAL

Cell culture and transfection

Human CRC cell lines SW620 (CCL-227) and HCT116 (CCL-247EMT) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), cultured in RPMI-1640 medium containing 10 % FBS, and placed in an incubator. The reagents used in the incubation were purchased from Thermo Fisher Scientific (Shanghai, China).

Experimental cells were infected with a lentiviral vector, pLVX-ZsGreen-miRNA-Puro (VT2242, YouBio, Hunan, China), that mediates overexpression of miR-143-5p (LV-miR-143-5p) or a negative control sequence, as well as the pcDNA3.1-HMGA2 vector or an empty vector. Lipofectamine® 3000 (L3000008, Solarbio Life Sciences, Beijing, China) was used for transfection according to the instructions. Stable clones were selected using 2 μg/mL puromycin (Thermo Fisher Scientific). miR-143-5p mimics and inhibitors, as well as the corresponding negative controls, were purchased from RiboBio (Guangzhou, China) and transfected into cells by Lipofectamine 2000 reagent (Solarbio Life Sciences) for subsequent experiments.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of SW620 and HCT116 cells was extracted using TRIzol reagent (Thermo Fisher Scientific). For reverse transcription of miRNA, a Mir-X™ miRNA qRT-PCR SYBR® Kit (638316) was used. For mRNA reverse transcription, a PrimeScript™ RT kit (RR037B) was used while qPCR analysis was performed with SYBR Premix Ex TaqII (RR820A). The kits used in the procedure were purchased from TAKARA (Beijing, China). Expression of miR-143-5p or *HMGA2* was normalized to *U6* or *GAPDH* with the $2^{-\Delta\Delta Ct}$ method [9]. The qPCR primer sequences used during the experiments are detailed in Table 1.

Table 1: Primers used for qRT-PCR

Gene	Primer sequence (5`-3`)
miR-143-5p	F: TGGTTCGTGGGGTCCAGTTTTCCCAG R: GTGTCGTGGAGTCGGCAATTC
U6	F: CTTCGGCAGCACATATACTAAAAT R: CGCTTCACGAATTTGCGTGTCAT
HMGA2	F: GGGCGCCGACATTCAAT R: ACTGCAGTGTCTTCTCCCTTCAA
GAPDH	F: TCAAGGCTGAGAACGGGAAG R: TGGACTCCACGACGTACTCA

Western blotting analysis

Cell lysates were obtained from SW620 and HCT116 cells treated with lysis buffer (P0013K) that was purchased from Beyotime (Jiangsu, China). Equal amounts of proteins were separated on 12% SDS-PAGE and transferred to PVDF membranes (YA1701, Solarbio Life Sciences), followed by closure in TBST containing 3% BSA. Membranes were incubated overnight at 4ºC with primary antibody and GAPDH (ab9485, dilution of 1:2500). Horseradish peroxidase-conjugated anti-rabbit secondary antibody (ab205718, dilution of 1:5000) was added and incubation was continued for 1h. Finally, protein bands were observed by incubation with chemiluminescent reagents. All antibodies in the above process were purchased from Abcam (Cambridge, UK). Primary antibody information is detailed in Table 2.

Cell viability assay

Cell counting kit-8 (CCK-8) (C0039) was purchased from Beyotime to assay SW620 and HCT116 cell viability. Transfected cells at a density of 1 × 10^3 cells/well were transferred into 96-well plates and grown overnight at room temperature. CCK8 reagent (10 μL) was added

to each well at the indicated time, and incubation was continued for 1 h. The absorbance at 450 nm was read using a multi-mode enzyme marker (Thermo Fisher Scientific).

Table 2: Primary antibodies used in western blot analysis

Proteins ratio	Item no.	Dilution
E-cadherin	ab40772	1:10000
ZO-1	ab276131	1:1000
N-cadherin	ab76011	1:10000
Vimentin	ab92547	1:2000
HMGA2	ab207301	1:1000

Proliferation assay

Transfected cells were transferred into 6-well plates at a density of 1 × 10^3 cells/well and maintained for 2 weeks. The cells were fixed using methanol/acetone (1:1) and stained using 0.1 % crystal violet. Images were taken, and the colonies that formed were counted with Image J software.

Migration assay

SW620 and HCT116 cells were transferred into culture inserts in multi-well plates (80206, Ibidi, Martin Reid, Germany) for 24 h. The culture inserts were raised with forceps, and fresh media was added. After 24 h, the culture inserts were removed to form 500-μm cell-free gaps. Images were acquired at 0 and 24 h under an inverted microscope, and wound widths were calculated with Image J software.

Invasion assay

A transwell assay was used to estimate the invasive ability of SW620 and HCT116 cells. Eight-μm transwell chambers (Corning, Kennebunk, USA) were coated with diluted Matrigel (BD Biosciences, CA, USA), and CRC cells pretreated with serum-free medium were transferred into the upper chamber at a density of 5 × 10^4. Serum-free medium was added to the upper chamber, and medium containing 10 % FBS was added to the lower chamber. After 48 h of incubation, cells were fixed with methanol and stained with 0.1 % crystal violet solution. Cells in the upper chamber were wiped off with a cotton swab and counted under an inverted microscope (Leica, Wetzlar, Germany).

Dual luciferase reporter gene experiments

The binding sites for miR-143-5p and *HMGA2* were predicted at the TargetScan website. Wild-type (WT) or mutant (MUT) fragments of *HMGA2* 3`-UTR containing the miR-143-5p binding site were inserted into the pmirGLO vector (VT1439, YouBio) to construct the luciferase reporter plasmid. SW620 cells were transferred into 24-well plates and co-transfected with the constructed plasmids and miRNAs (miR-143-5p mimic, miR-143-5p inhibitor, or negative controls). After 48 h of warming, luciferase activity was measured using the Dual Luciferase Reporter Gene Assay System (Promega) and normalized to Renilla luciferase internal control activity.

Statistical analysis

The experiments were independently repeated three times. Data were analyzed using SPSS 22.0 (SPSS Inc, Chicago, IL, US). All experimental data are expressed as mean ± SD. Student's t tests were performed to measure the differences between two groups, and $p < 0.05$ was considered statistically significant.

RESULTS

Upregulation of miR-143-5p expression inhibits proliferation of CRC cells

The miR-143-5p expression of two CRC cell lines was quantified using qRT-PCR, which showed that miR-143-5p expression was significantly higher in SW620 cells with high malignancy than in HCT116 cells, indicating that the expression level of miR-143-5p may be related to the malignancy of CRC cells (Figure 1 A). To confirm whether miR-143-5p affects the malignant process of CRC, we overexpressed miR-143-5p (Figure 1B) and analyzed the effect of miR-143-5p on the proliferation of CRC cells using CCK-8 and clone formation assays (Figure 1 C and D). The results showed that overexpression of miR-143-5p significantly inhibited the viability and proliferative capacity of SW620 and HCT116 cells.

Upregulation of miR-143-5p expression inhibits CRC cell migration, invasion, and EMT

The results of the wound healing assay showed that the gap for SW620 and HCT116 cells transfected with LV-miR-143-5p was wider than that of cells transfected with LV-NC after 24 h and that the degree of wound healing was reduced (Figure 2 A). Results of the transwell assay showed that the invasion numbers for SW620 and HCT116 cells transfected with LV-miR-143-5p were significantly lower than the invasion number of the LV-NC group (Figure 2 B). The inhibitory effect of miR-143-5p on the

migratory and invasive ability of CRC cells was confirmed.

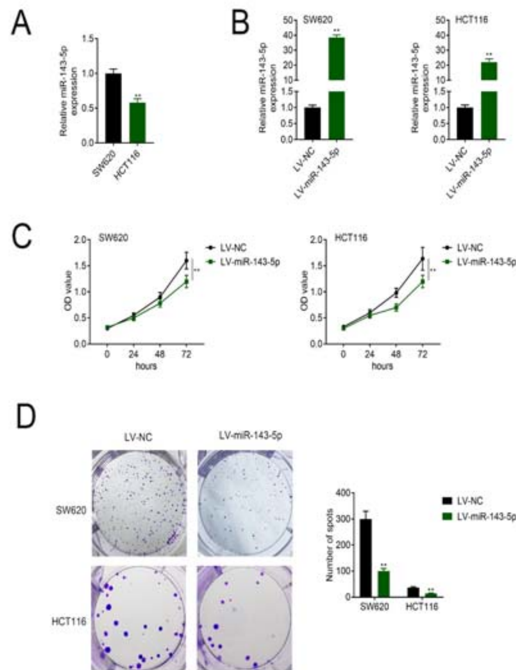

Figure 1: Effect of miR-143-5p overexpression on the proliferation of CRC cells. (A) Relative expression of miR-143-5p in SW620 and HCT116 cells was examined by qRT-PCR. (B) qRT-PCR determined the transfection efficiency of LV-miR-143-5p in CRC cells. (C) CCK-8 assay measured viability of CRC cells after transfection with LV-miR-143-5p. (D) Colony formation assay detected the proliferation of CRC cells after transfection with LV-miR-143-5p; ** $p < 0.01$

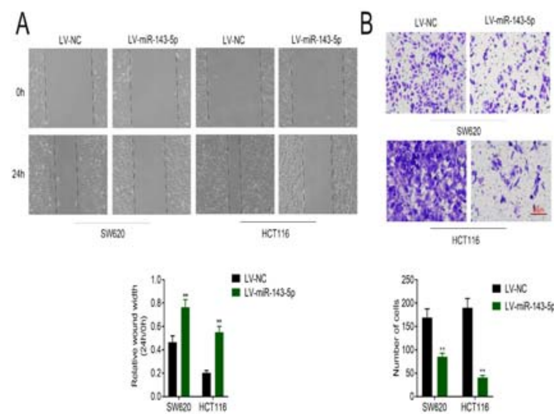

Figure 2: Effect of miR-143-5p overexpression on migration and invasion of CRC cells. (A) Wound healing assay to analyze the migration ability of CRC cells with or without miR-143-5p overexpression. (B) Transwell assay to analyze the invasion ability of CRC cells with or without miR-143-5p overexpression; ** $p < 0.01$

Upregulation of miR-143-5p expression inhibits EMT in CRC cells

After miR-143-5p expression was upregulated, a significant increase in the expression of E-cadherin and ZO-1 was detected in SW620 and HCT116 cells, while vimentin and N-cadherin expression was significantly decreased (Figure 3). This demonstrated the inhibitory effect of miR-143-5p on the EMT process in CRC cells.

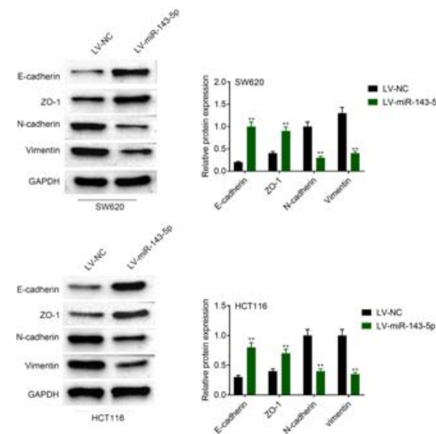

Figure 3: Effect of miR-143-5p overexpression on EMT in CRC cells. Western blot to analyze the expression levels of E-cadherin, ZO-1, vimentin, and N-cadherin in SW620 and HCT116 cells transfected with LV-NC or LV-miR-143-5p; **$p < 0.01$

***HMGA2* is a downstream target gene of miR-143-5p**

The potential target gene of miR-143-5p was predicted with the bioinformatics database TargetScanHuman7.2. *HMGA2* 3`-UTR was revealed to have a binding site to miR-143-5p, and an *HMGA2* 3`-UTR mutant fragment with a miR-143-5p binding site was obtained by targeted mutagenesis (Figure 4 A). A subsequent dual luciferase reporter gene assay revealed no significant change in luciferase activity in the mutant group co-transfected with miR-143-5p mimic or inhibitor, while luciferase activity was significantly reduced or increased, respectively, in the WT group (Figure 4 B). This indicates that miR-143-5p may regulate *HMGA2* expression by targeting *HMGA2* 3`-UTR. To further verify this targeting-regulation relationship, SW620 cells were transfected with miR-143-5p mimic, miR-143-5p inhibitor, and their respective controls, and the protein expression levels of HMGA2 were analyzed with western blot. As shown (Figure 4 C), up/downregulation of miR-143-5p significantly inhibited/increased the expression level of *HMGA2*.

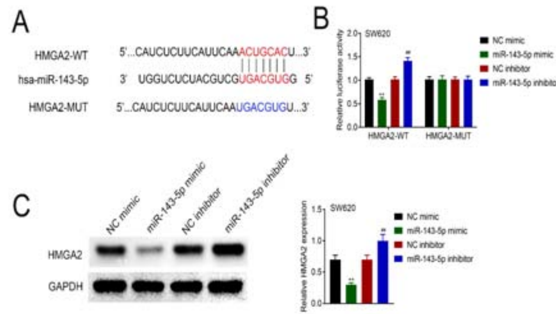

Figure 4: miR-143-5p downregulated HMGA2 expression by targeting *HMGA2* 3`-UTR. (A) Predicted binding sites of miR-143-5p to *HMGA2* 3`-UTR and mutant binding sites. (B) The luciferase activities of miR-143-5p mimic or inhibitor + *HMGA2*-WT or *HMGA2*-MUT were compared in the SW620 cell, respectively. (C) Western blot to analyze the protein expression changes of *HMGA2* in SW620 cells transfected with miR-143-5p mimic or inhibitor; ***p* < 0.01, compared to NC mimic. ## *p* < 0.01, compared to NC inhibitor

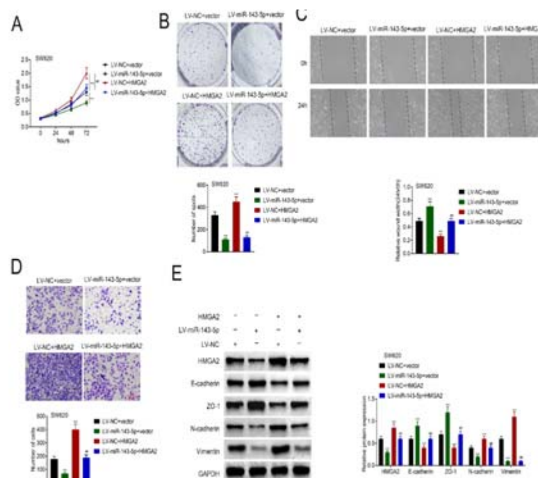

Figure 5: Upregulation of *HMGA2* reverses the inhibitory effect of miR-143-5p overexpression on CRC cell proliferation and metastasis. (A) CCK-8 assay used to analyze the viability of SW620 cells treated with different transfections. (B) Colony formation assay used to analyze the proliferation of SW620 cells in each group. (C) Wound healing assay used to detect the migration ability of SW620 cells in each group. (D) Transwell assay used to determine the invasive ability of SW620 cells in each group. (E) Western blot used to analyze the expression levels of EMT-related markers in each group of SW620 cells. ***P* < 0.01, compared to NC mimic; ##*p* < 0.01, compared to NC inhibitor

Upregulation of *HMGA2* reversed the effect of miR-143-5p overexpression on CRC cell proliferation and metastasis

In the final rescue assay, results from the CCK-8 and colony formation assays showed that elevated *HMGA2* levels significantly reversed the effect of overexpressed miR-143-5p on cell viability and proliferation (Figure 5 A and B). This reversal effect was consistent with the results of the wound healing and transwell invasion assays, which showed that upregulation of HMGA2 reversed the inhibitory effect of miR-143-5p on cell migration and invasion (Figure 5 C and D). Western blot revealed that miR-143-5p overexpression significantly increased levels of E-cadherin and ZO-1, but decreased levels of N-cadherin and vimentin. With the restoration of *HMGA2* expression, the regulatory effect of miR-143-5p overexpression on the levels of the above-mentioned related proteins were reversed (Figure 5E), confirming that upregulation of *HMGA2* reversed the inhibitory action of miR-143-5p on CRC cell metastasis by stimulating the EMT process in CRC cells.

DISCUSSION

MiR-143-5p is reportedly involved in the regulation of tumor growth and metastasis through multiple biological pathways. Several CRC-related studies have identified miR-143-5p as a potential prognostic marker. A study on microRNA characterization has shown that a classifier containing six miRNAs, including miR-143-5p, which was constructed based on miRNA microarrays and tissue samples from stage II CRC patients, is a valid prognostic indicator of disease recurrence in stage II CRC patients [10]. Caritg *et al* further identified miR-143-5p, miR-103a-3p, and miR-215 as a three-miRNA risk scoring model based on 6 miRNA classifiers, adding prognostic information of value to the clinical features of stage II CRC [8]. These studies confirm the clinical value of miR-143-5p as a prognostic marker for CRC, and the present study built on this to elaborate the role of miR-143-5p in the regulation of CRC progression.

In this study, a series of cell biological function assays confirmed the inhibitory effects of miR-143-5p on proliferation, migration, invasion, and EMT in SW620 and HCT116 cells. The results were consistent with the antitumor effect elicited by miR-143-5p in other reported cancers. EMT, a process that loosens intercellular adhesion complexes and confers stronger migratory and invasive properties to cells, is correlated with tumorigenesis, invasion, and therapeutic resistance [11,12]. In esophageal cancer, miR-143-5p attenuates metastatic potential by targeting LAMP3 to inhibit EMT [13]. The study presented herein confirmed that upregulation of miR-143-5p significantly inhibited EMT in SW620 and HCT116 cells, strongly supporting the notion that miR-143-5p may affect CRC cell metastasis by suppressing the expression of EMT markers.

In the investigation of the anti-tumor effect of miR-143-5p in CRC development, identification of the downstream target genes of this miRNA was critical. miRNAs are dysregulated in response to multiple factors and affect the malignant phenotype of cancer cells at the post-transcriptional regulatory level by binding to the 3`-UTR of the encoding RNA [14,15]. Bioinformatics predicts that *HMGA2*, a target of miR-143-5p, a gene located on chromosome 12q14-15, is a transcriptional regulator that controls mesenchymal differentiation and the induction of benign mesenchymal tumors. Dysregulated expression is usually closely associated with tumorigenic transformation [16-18]. A CRC-related clinical study has indicated that ectopic expression of *HMGA2* induces EMT, which is critical for CRC metastasis [19].

In the rescue experiments, upregulation of *HMGA2* reversed the inhibitory effects of miR-143-5p as a tumor suppressor miRNA in CRC on proliferation, migration, invasion, and EMT of SW620 cells. The pro-carcinogenic effect of HMGA2 exhibited in CRC in this study was consistent with what has been reported, including the promotion of EMT in CRC cells by HMGA2. That is, miR-143-5p inhibited proliferation, invasion, and EMT of CRC cells, in part through downregulation of *HMGA2* expression.

CONCLUSION

This study demonstrates the inhibitory effect of miR-143-5p in CRC development and, for the first time, reports the molecular mechanism of the antitumor effect of miR-143-5p in CRC via the inhibition of *HMGA2*. This finding will aid in the exploration of therapeutic targets for CRC that may lead to the development of more effective clinical treatment strategies.

DECLARATIONS

Conflict of interest

No conflict of interest to disclose with regard to this work.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xiuqing Li and Hui Zhang designed the study and supervised data collection, Tao Cui analyzed and interpreted the data, and Youshan Wu and Shougang Wang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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REFERENCES

1. *Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: CA-Cancer J Clin 2020; 70(1): 7-30.*
2. *Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116(2): 281-297.*
3. *Ambros V. The functions of animal microRNAs. Nature 2004; 431(7006): 350-355.*
4. *Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA et al. MicroRNA expression profiles classify human cancers. Nature 2005; 435(7043): 834-838.*
5. *Li Y, Ma X, Li J, He S, Zhuang J, Wang G, Ye Y, Xia W. LncRNA gas5 regulates granulosa cell apoptosis and viability following radiation by x-ray via sponging miR-205-5p and Wnt/β-catenin signaling pathway in granulosa cell tumor of ovary. Trop J Pharm Res 2020; 19(6): 1153-1159.*
6. *Barisciano G, Colangelo T, Rosato V, Muccillo L, Taddei ML, Ippolito L, Chiarugi P, Galgani M, Bruzzaniti S, Matarese G et al. miR-27a is a master regulator of metabolic reprogramming and chemoresistance in colorectal cancer. Br J Cancer 2020; 122(9): 1354-1366.*
7. *Clancy C, Joyce MR, Kerin MJ. The use of circulating microRNAs as diagnostic biomarkers in colorectal cancer. Cancer Biomark 2015; 15(2): 103-113.*
8. *Caritg O, Navarro A, Moreno I, Martínez-Rodenas F, Cordeiro A, Muñoz C, Ruiz-Martinez M, Santasusagna S, Castellano JJ, Monzó M. Identifying High-Risk Stage II Colon Cancer Patients: A Three-MicroRNA-Based Score as a Prognostic Biomarker. Clin Colorectal Cancer 2016; 15(4): e175-e182.*
9. *Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008; 3(6): 1101-1108.*

10. *Zhang JX, Song W, Chen ZH, Wei JH, Liao YJ, Lei J, Hu M, Chen GZ, Liao B, Lu J et al. Prognostic and predictive value of a microRNA signature in stage II colon cancer: a microRNA expression analysis. Lancet Oncol 2013; 14(13): 1295-1306.*
11. *Pastushenko I, Blanpain C. EMT Transition States during Tumor Progression and Metastasis. Trends Cell Biol 2019; 29(3): 212-226.*
12. *Suarez-Carmona M, Lesage J, Cataldo D, Gilles C. EMT and inflammation: inseparable actors of cancer progression. Mol Oncol 2017; 11(7): 805-823.*
13. *Yang C, Shen S, Zheng X, Ye K, Sun Y, Lu Y, Ge H. Long noncoding RNA HAGLR acts as a microRNA-143-5p sponge to regulate epithelial-mesenchymal transition and metastatic potential in esophageal cancer by regulating LAMP3. Faseb J 2019; 33(9): 10490-10504.*
14. *Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15(8): 509-524.*
15. *Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annu Rev Pathol 2014; 9(287-314.*
16. *Fusco A, Fedele M. Roles of HMGA proteins in cancer. Nat Rev Cancer 2007; 7(12): 899-910.*
17. *Zaidi MR, Okada Y, Chada KK. Misexpression of full-length HMGA2 induces benign mesenchymal tumors in mice. Cancer Res 2006; 66(15): 7453-7459.*
18. *Ligon AH, Moore SD, Parisi MA, Mealiffe ME, Harris DJ, Ferguson HL, Quade BJ, Morton CC. Constitutional rearrangement of the architectural factor HMGA2: a novel human phenotype including overgrowth and lipomas. Am J Hum Genet 2005; 76(2): 340-348.*
19. *Wang X, Liu X, Li AY, Chen L, Lai L, Lin HH, Hu S, Yao L, Peng J, Loera S et al. Overexpression of HMGA2 promotes metastasis and impacts survival of colorectal cancers. Clin Cancer Res 2011; 17(8): 2570-2580.*