ORIGINAL ARTICLE

Expression level of long noncoding RNA NKILA-miR103-miR107 inflammatory axis and its clinical significance as potential biomarker in patients with colorectal cancer

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Background: Inflammatory cytokines have been observed in colorectal cancer (CRC) tissues and can promote the susceptibility to metastasis of CRC cells. Diverse regulatory mechanisms of long ncRNAs (lncRNAs) and microRNAs (miRNAs) involved in the inflammatory responses are associated with tumor progression. The aim of this research was to investigate the expression level of the nuclear factor-kappa B interacting lncRNA (NKILA)-miR103-miR107 regulatory axis and its clinical significance as a potential biomarker in patients with CRC. **Materials and Methods:** In the present study, we investigated the expression levels of miR103, miR107, and NKILA in 21 paired CRC tissues and corresponding adjacent tissues, using real-time polymerase chain reaction technique. Receiver operating characteristic (ROC) curve was used to analyze the prognostic value of biomarkers and to compare their predictive value. **Results:** It was found that the expression level of miR103 was significantly increased with the development of CRC (cancerous vs. corresponding normal tissues; 2.29 ± 1.65 vs. 1.16 ± 0.64, $P = 0.003$). Moreover, miR107 was upregulated in CRC tissues compared with paired normal tissues (2.1 ± 1.4 vs. 1.25 ± 0.83, $P = 0.005$), while NKILA displayed an opposite expression pattern versus miR103/107, but it was not statistically significant (3.69 ± 5.2 vs. 4.35 ± 5.99, $P > 0.05$). The ROC analysis demonstrated that miR103 had the best diagnostic ability performance with area under curve of 0.723 (0.545–0.901). **Conclusion:** We identified miR103/107 as tumor-promoting miRNAs with diagnostic value in cancer patients and presumptive negative regulators of NKILA, a potential cancer metastatic suppressor. Strategies that disrupt this regulatory axis might block CRC progression.

Key words: Colorectal cancer, long non-coding RNA, nuclear factor-kappaB interacting lncRNA, miR-103a-3p, miR-107

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INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the fourth most common cause of cancer deaths worldwide, with over 1.2 million new cases each year.[1] Despite the advancement of cancer therapy modalities, including surgery, radiotherapy, chemotherapy, and targeted therapy, the 5-year overall survival rate of CRC patients has not changed dramatically during the past decades.[2,3] The identification of novel molecular biomarkers and development of effective therapeutic strategies are needed for CRC patients.[4,5] Numerous molecular mechanisms may be involved in the treatment failure and poor patient prognosis.[6] Recent evidence point to

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a relationship between the clinical outcome of patients and genetic heterogeneity within the patient's cancer cells.[2,7]

In the human genome sequencing project, it has been found that most of the genome is transcribed into RNAs with absent or limited protein-coding potential.[1,8] Mechanistic roles of noncoding RNAs (ncRNAs) have been recently demonstrated in the tumorigenesis and the development of therapeutic resistance.[1,2] The study of genes that produce short or long ncRNA transcripts without an apparent open reading frame has revealed that the complexity of cancer cell genetics is much higher than initially expected.[2]

Long ncRNAs (lncRNAs) and microRNAs (miRNAs) are two major classes of the ncRNAs family. They have critical roles in various biological processes. At present, their diverse regulatory mechanisms in cancer cells have gained increasing attention.[9] While much is known about miRNAs, little exists regarding the biology and function of lncRNAs. lncRNAs are a group of large and heterogeneous ncRNAs that regulate expression at the transcriptional and posttranscriptional level, mainly through an interaction with a variety of RNA such as miRNAs.[2,10] Along this line, many studies have demonstrated that miRNA-lncRNA interactions are closely linked to the occurrence and development of cancers.[11]

Constant activation of nuclear factor kappa B (NF-κB) is induced by inflammatory cytokines in CRC tissues. Indeed, NF-κB is a pivotal link between CRC and inflammation, and it is found to be overexpressed in nearly all CRC tissues and cell lines.[12,13] The growing evidence clearly points that NF-κB interacting lncRNA (NKILA) regulates inflammation and impedes cancer cell migration by attachment to NF-κB/IκB complex and directly blocking the phosphorylation of IκB and NF-κB activation.[14-16] More recently, crosstalk between NKILA and miR103/107 is demonstrated in breast cancer which was related to tumor progression. NKILA and miR103/107 inflammatory axis may also be involved in CRC cancer biology. However, the expression levels of the NKILA-miR103/107 network and its clinical significance in CRC remains elusive so far. The aim of this research was to investigate the expression level of this regulatory axis and its clinical significance as a potential biomarker in patients with CRC.

MATERIALS AND METHODS

Ethics

Written informed consent was obtained from each patient, and all procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Tehran University of Medical Sciences and with the Helsinki declaration of 1975, as revised in 2000.

Selection and description of participants

In this analytical case–control study, fresh surgical specimens of cancer tissue and adjacent nontumor tissues were obtained from 21 patients with primary CRC, who underwent surgery without preoperative treatment at the endoscopy clinic of Masood and Shariati hospital in Tehran, Iran, from May 2018 to January 2019. All enrolled patients met the following criteria for inclusion: (1) not received neoadjuvant chemoradiation, (2) histological confirmed adenocarcinoma, and (3) underwent curative R0 resection. Patients were excluded from the study if they had died or had insufficient clinicopathological data. The patients were diagnosed by pathological tests and imaging examinations.

Total RNA extraction and complementary DNA synthesis

Before RNA extraction, freshly harvested tissue samples were immediately placed and stabilized in 10 volumes of RNAlater™ solution (Qiagen, Hilden, Germany) for 1 or 4 weeks at 4°C. Total RNA was isolated with RNeasy Mini spin column procedure (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. It provides fast and efficient purification of both large (>200 nt) and small RNA species (10–200 nt) including miRNAs. The complementary DNA (cDNA) synthesis for miRNAs and lncRNAs were performed using the miScript II Reverse Transcription Kit (Qiagen, Hilden, Germany) or the PrimeScript™ 1st strand cDNA Synthesis Kit (TaKaRa, Dalian, China) respectively, according to the manufacturer's instructions. Quantity and quality of all RNA samples were assessed by measuring OD at 260/280 nm between 1.8 and 2.0 using the Thermo Scientific™ NanoDrop™ One Spectrophotometer (Life Technologies/ThemoFisher Scientific, Hudson, NH).

Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (RT-PCR) analyses for lncRNA NKILA were performed using the StepOnePlus RT-PCR System (Thermo Fisher Scientific, USA) according to the following protocol: After a preliminary denaturing step at 95°C for 15 min, PCR amplification was performed for 40 cycles: 15 sec denaturing at 95°C, 20 s at 59°C as annealing temperature, and 20 sec extension at 72°C, with a final 5 min step at 72°C. Each reaction was carried out in 20 μL reactions containing 4 μL × 5 HOT FIREPol EvaGreen qPCR Mix Plus (ROX) (Solis BioDyne, Tartu, Estonia), 1 μL of each primer (final concentration 10 μM), 12 μL RNase-free water and 2 μL cDNA. Normalized expression levels were calculated using the expression of GAPDH gene as the normalization reference. Following primers were used in PCR reactions: 5′-AACCAAACCTACCCACAACG-3′ (forward) and 5′-ACCACTAAGTCAATCCCAGGTG-3′ (reverse) for NKILA; 5′-ATCACCATCTTCCAGGAGCGA-3′ (forward) and 5′-CCTTCTCCATGGTGGTGAAGAC-3′ (reverse) for GAPDH.

Real-time miRNA detection for miR103 and 107 was performed on the StepOnePlus Real-Time PCR thermocycler using miScript SYBR Green PCR Kit (Qiagen, Hilden, Germany, Cat. No: 218076), with 25 μΛ mixtures containing 12.5 μΛ of × 2 QuantiTect SYBR Green PCR Master Mix, 2.5 μΛ of × 10 miScript Universal primer, 2.5 μΛ of × 10 miScript Primer Assay for miR103 (Qiagen, Hilden, Germany, Cat. No: MS00031241) and 107 (Qiagen, Hilden, Germany, Cat. No: MS00003409), 5.5 μΛ of RNase-free water, and 2 μΛ of template cDNA. The housekeeping miRNA SNORD68 (Qiagen, Hilden, Germany, Cat. No: MS00033712) was used as the endogenous control and miR-103/107 cycle threshold (Ct) values were normalized to SNORD68 Ct values. Cycling conditions for real-time PCR were 95°C for 15 min followed by 40 cycles of 94°C for 15 s, 55°C for 30 s, and 70°C for 30 s. At the end of the 40 cycles, a melt-curve analysis was used to evaluate PCR specificity. Geometric means of Ct values of reference genes were used in the analysis. For each of the samples, Ct differences between target and reference genes were calculated and Ct values were processed using $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Statistical analyses were performed by SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA), and graphs were generated using GraphPad Prism version 5.0 (GraphPad Software, Inc., San Diego, CA, USA). The Chi-square and mean comparison tests were performed to explore the associations between the NKILA-miR103-107 axis expression level and the clinicopathological characteristics. Statistical analyses were performed by comparing the Ct value of each experimental group with that of the control group by the Mann–Whitney U-test. A receiver operating characteristic (ROC) study was also performed to evaluate the prognostic value of biomarkers and to compare their predictive value. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Relationship between lncRNA NKILA-miR103/107 axis expression and clinical features of colorectal cancer patients

The study consisted of 21 patients with CRC and included 10 males (47.6%) and 11 females (52.4%) with a mean age of 64.44 ± 8.03 years (range: 50–85 years) and 63 ± 8.36 years (range: 52–82 years), respectively. To explore the correlation of lncRNA NKILA-miR103/107 expression levels with clinicopathological features in CRC patients, we divided the 21 CRC patients into a high gene expression group and a low expression group according to the median value of relative gene expression. The majority of patients were in a worse histological differentiation, including 56.25% poor grade patients. miR103 expression in CRC tissues varied, and was associated with histological grade ($P = 0.042$), but was not correlated (all $P > 0.05$) with other available clinicopathological characteristics.

LncRNA NKILA displayed an opposite expression pattern versus miR103/107 in colorectal cancer tissues

Here, we examined the relative expression pattern of lncRNA NKILA-miR103/107 by quantitative reverse transcription-polymerase chain reaction in a total of 21 paired CRC and matched adjacent noncancerous tissues. We found that, as shown in Figure 1a, the expression level of miR103 was higher in malignant colorectal tissues compared with adjacent normal tissues (cancer tissues vs. corresponding noncancerous tissues; 2.29 ± 1.65 vs. 1.16 ± 0.64, $P = 0.0032$). The results of the present study also reveal that miR107 was upregulated in CRC tissues compared with paired nontumor tissues [2.1 ± 1.4 vs. 1.25 ± 0.83, $P = 0.005$, Figure 1b]. Furthermore, we showed that NKILA was downregulated in CRC cancer tissues than that in matched adjacent noncancerous tissues and displayed an opposite expression pattern versus miR103/107, but it was not statistically significant [3.69 ± 5.2 vs. 4.35 ± 5.99, $P > 0.05$, Figure 2].

Receiver operating characteristic curves of single tumor markers in colorectal cancer patients

We analyzed the ROC curves of tissue miR103, miR107, and NKILA in CRC patients [Figure 3]. The miR103 area under curve (AUC) was 0.723, with 95% confidence interval (CI) 0.545–0.901 ($P = 0.026$). The miR107 AUC was 0.720 (95% CI 0.547–0.892, $P = 0.029$) and the NKILA AUC was 0.564 (95% CI 0. 367–0. 761, $P > 0.05$) [Table 1]. When the cutoff value for miR103 was determined by the maximum value of the Youden index, the sensitivity and specificity for were 71.1% and 77.2%, respectively. When the cutoff value for miR107 was selected, the sensitivity and specificity for miR107 were 53.1% and 88.2%, respectively. The ROC curve demonstrated that miR103 had the best diagnostic ability performance with the highest AUC. miR107 seemed to be inferior to miR103 in sensitivity and AUC when detecting CRC; however, its specificity was all the highest among the three markers.

DISCUSSION

Inflammation is demonstrated to be one of the main predisposing factors of CRC.[7,17] Pro-inflammatory

Table 1: Comparison of average receiver operating characteristic curves

Variables	Area	Significant*	Confidence interval 95%	
			Lower bound	Upper bound
NKILA	0.564	0.524	0.367	0.761
miR103	0.723	0.026	0.545	0.901
miR107	0.720	0.029	0.547	0.892

NKILA=NF-kB interacting long ncRNA, *Significant: $P < 0.05$

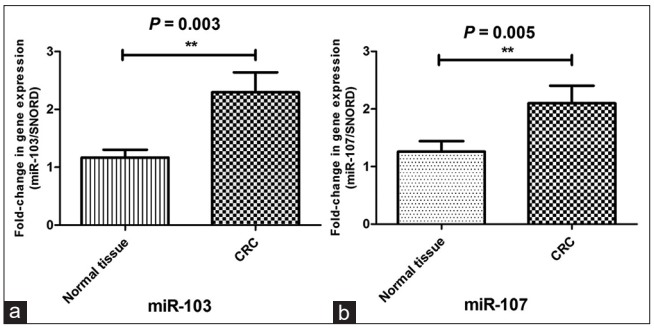

Figure 1: miR103 (a) and miR107 (b) are upregulated in colorectal cancer. Relative miR103/107 expression levels in 21 paired colorectal cancer tissues and adjacent nontumor tissues was assessed by quantitative reverse transcription polymerase chain reaction analysis. Small nucleolar RNA, C/D box 68 (SNORD68) was used as an internal control. The data represents the mean ± standard error of the mean

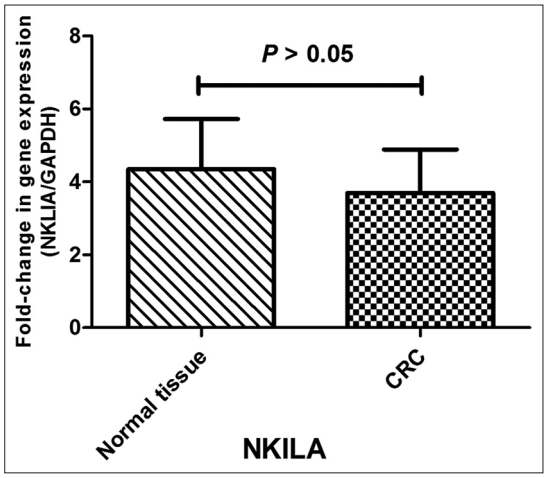

Figure 2: Expression of long noncoding RNA (lncRNA) NKILA in colorectal cancer tissues. Relative expression of NKILA was determined by quantitative reverse transcription polymerase chain reaction analysis. GAPDH was used as an internal control. The data represents the mean ± standard error of the mean ($P < 0.05$)

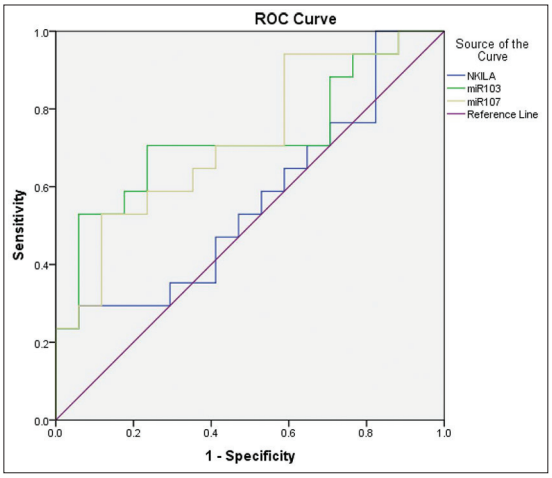

Figure 3: Receiver operating characteristic curve analysis of mucosal gene expression of lncRNA NKILA-miR103-miR107 axis in patients with colorectal cancer

pathways, including NF-κB, IL-6/STAT3, and IL-23/Th17, induce tumorigenesis by prompting the production of inflammatory mediators, upregulating the expression levels of antiapoptotic proteins, and stimulating cell proliferation as well as epithelial-to-mesenchymal transition (EMT).[18]

Accumulating evidence suggest lncRNA-miRNA pairs have the ability to interact with proteins, especially critical signaling pathway transcription factors that promote cancer growth.[14,19] Little is known about the noncoding RNA signaling networks involved in cancer initiation and progression.[18,20,21] The identification of functional interactions among miRNAs and lncRNAs is valuable for understanding RNA signaling networks involved in cancer development.[22] Numerous miRNAs have been found to play important regulatory roles in either promoting or suppressing cancer progression.[23,24] In a previous study, cytoplasmic miR103/107 have been shown to promote metastasis of CRC by targeting the metastasis suppressors DAPK and KLF4 for degradation.[25] On the other hand, profiling of the high-metastatic breast cancer cells demonstrated that NKILA is one of the most downregulated lncRNA in cancer metastasis[26] and higher expression of miR103/107 was found to increase cancer cell proliferation/survival and induce tumor metastasis through the targeted degradation of NKILA in breast cancer.[26,27] In breast cancer, NKILA has been recently shown to be regulated by specific miRNAs especially miR103 and miR107 and its expression level was much lower in high-metastatic than low-metastatic cancer cells.[26] Here, we investigated the expression levels of NKILA-miR103-miR107 regulatory axis in patients with CRC. We found that miR103/107 display an opposite expression pattern versus NKILA, in such a way that miR103/107 are dramatically upregulated and NKILA expression is decreased in CRC tissues compared with adjacent nontumor tissues. The novel identified relationship can be critical for the formation of tumor and proposes a new role for miR103/107 in the regulation and function of NKILA in human colon cancer.

Recently, several lncRNA-miRNA networks have been documented to promote tumor progression and metastasis through different mechanisms. Accumulating evidence suggest that the best-characterized functional mechanism of lncRNA-miRNA interaction is by competing endogenous RNAs (ceRNAs). In this regard, lncRNAs could function as miRNA sponges, reducing their regulatory influence on mRNAs. On the other hand, miRNAs can regulate lncRNAs by targeting them for degradation through the RNA-induced silencing complex,[28] known as "miRNA-triggered lncRNA decay."[22] This biological process adds another layer of complexity in the miRNA–target interaction network.[29] Along this line, a recent study by Liu *et al.* have identified that negative regulation of NKILA by miR103/107 is necessary for breast cancer toumorogenesis.[26] NKILA expression was significantly increased in miR103/107 knockout cancerous

cells and was dropped sharply after overexpression of miR103/107, showing that decreased NKILA expression releases suppression of cancer stem cells and leads to higher tumorigenicity. Mechanistic studies revealed the potential mechanism of action for NKILA-target network, as NKILA directly interact with the transcription factor NF-κB by inhibiting the IKK phosphorylating sites of IκB as well as the induction of EMT process.[26,30] NKILA may be a potential downstream target of miR103/107 in cancer cells as recent evidence have shown that miR103/107 physically interacts with NKILA and reduces the expression level of NKILA in breast cancer cells.[26] These results as well as our findings of opposite expression levels of NKILA and miR103/107 in colon cancer tissues compared to healthy colon tissues indicate that NKILA-miR103/107 regulatory axis is involved in CRC progression.

In the present study, we compared the ROC curves of the three tumor markers, NKILA, miR103, and miR107, in patients with CRC. miR103 seemed to be the best in its predictive efficacy, with the highest sensitivity and AUC of the three. However, the specificity of miR103 was low, which resulted in a false-positive rate being too high. miR103 was recently proved to have a diagnostic value and function as a prometastatic miRNA in CRC.[31,32] Similarly, we found its diagnostic value in CRC. miR107 appeared to be inferior to miR103 in sensitivity and AUC average when detecting CRC; however, its specificity was higher than the other two markers. Further studies are needed to evaluate the potential of other cancer-related miRNA-lncRNA networks as a valuable biomarker of the progression and the prognosis in CRC.

CONCLUSION

We studied NF-kB interacting lncRNA (NKILA)-miR103-miR107 regulatory axis in patients with CRC and its contributions to tumor development. Our results suggest that NKILA may be considered as a new potential downstream regulator of miR103/107 in CRC, where recent evidence have shown that miR103/107 physically interact with NKILA and reduce the expression level of NKILA in cancer tissue. In addition, miR103/107 were found to have a diagnostic value in CRC patients and may act as proper biomarkers due to their abundance and stability. The development of miR103/107-based therapeutic strategies for the upregulation of NKILA may provide a new and promising alternative therapeutic approach for colon treatment.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Li X, Wang F, Sun Y, Fan Q, Cui G. Expression of long non-coding RNA PANDAR and its prognostic value in colorectal cancer patients. Int J Biol Markers 2017;32:e218-e223.
2. Malek E, Jagannathan S, Driscoll JJ. Correlation of long non-coding RNA expression with metastasis, drug resistance and clinical outcome in cancer. Oncotarget 2014;5:8027-38.
3. Yin DD, Liu ZJ, Zhang E, Kong R, Zhang ZH, Guo RH. Decreased expression of long noncoding RNA MEG3 affects cell proliferation and predicts a poor prognosis in patients with colorectal cancer. Tumour Biol 2015;36:4851-9.
4. Punt CJ, Koopman M, Vermeulen L. From tumour heterogeneity to advances in precision treatment of colorectal cancer. Nat Rev Clin Oncol 2017;14:235-46.
5. Jalaeikhoo H, Zokaasadi M, Khajeh-Mehrizi A, Rajaeinejad M, Mousavi SA, Vaezi M, *et al*. Effectiveness of adjuvant chemotherapy in patients with stage II colorectal cancer: A multicenter retrospective study. J Res Med Sci 2019;24:39.
6. Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer 2009;9:489-99.
7. Javarsiani MH, Javanmard SH, Colonna F. Metastatic components in colorectal cancer. J Res Med Sci 2019;24:75.
8. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, *et al*. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 2009;458:223-7.
9. Xue M, Zhuo Y, Shan B. MicroRNAs, long noncoding RNAs, and their functions in human disease. Methods Mol Biol 2017;1617:1-25.
10. Dykes IM, Emanueli C. Transcriptional and post-transcriptional gene regulation by long non-coding RNA. Genomics Proteomics Bioinformatics 2017;15:177-86.
11. Zhang G, Pian C, Chen Z, Zhang J, Xu M, Zhang L, *et al*. Identification of cancer-related miRNA-lncRNA biomarkers using a basic miRNA-lncRNA network. PLoS One 2018;13:e0196681.
12. Huber MA, Beug H, Wirth T. Epithelial-mesenchymal transition: NF-kappaB takes center stage. Cell Cycle 2004;3:1477-80.
13. Yang J, Weinberg RA. Epithelial-mesenchymal transition: At the crossroads of development and tumor metastasis. Dev Cell 2008;14:818-29.
14. Huang W, Cui X, Chen J, Feng Y, Song E, Li J, *et al*. Long non-coding RNA NKILA inhibits migration and invasion of tongue squamous cell carcinoma cells via suppressing epithelial-mesenchymal transition. Oncotarget 2016;7:62520-32.
15. Lu Z, Li Y, Wang J, Che Y, Sun S, Huang J, *et al*. Long non-coding RNA NKILA inhibits migration and invasion of non-small cell lung cancer via NF-κB/Snail pathway. J Exp Clin Cancer Res 2017;36:54.
16. Bian D, Gao C, Bao K, Song G. The long non-coding RNA NKILA inhibits the invasion-metastasis cascade of malignant melanoma via the regulation of NF-κB. Am J Cancer Res 2017;7:28-40.
17. Wang T, Song P, Zhong T, Wang X, Xiang X, Liu Q, *et al*. The inflammatory cytokine IL-6 induces FRA1 deacetylation promoting colorectal cancer stem-like properties. Oncogene 2019;38:4932-47.
18. Anastasiadou E, Jacob LS, Slack FJ. Non-coding RNA networks in cancer. Nat Rev Cancer 2018;18:5-18.
19. Nair S. Current insights into the molecular systems pharmacology of lncRNA-miRNA regulatory interactions and implications in cancer translational medicine. AIMS Mol Sci 2016;3:104-24.
20. Wang J, Du Y, Liu X, Cho WC, Yang Y. MicroRNAs as regulator of signaling networks in metastatic colon cancer. Biomed Res Int

2015;2015:823620.
21. Qiu MT, Hu JW, Yin R, Xu L. Long noncoding RNA: An emerging paradigm of cancer research. Tumour Biol 2013;34:613-20.
22. Yoon JH, Abdelmohsen K, Gorospe M. Functional interactions among microRNAs and long noncoding RNAs. Semin Cell Dev Biol 2014;34:9-14.
23. Orang AV, Barzegari A. MicroRNAs in colorectal cancer: From diagnosis to targeted therapy. Asian Pac J Cancer Prev 2014;15:6989-99.
24. To KK, Tong CW, Wu M, Cho WC. MicroRNAs in the prognosis and therapy of colorectal cancer: From bench to bedside. World J Gastroenterol 2018;24:2949-73.
25. Chen HY, Lin YM, Chung HC, Lang YD, Lin CJ, Huang J, *et al*. miR-103/107 promote metastasis of colorectal cancer by targeting the metastasis suppressors DAPK and KLF4. Cancer Res 2012;72:3631-41.
26. Liu B, Sun L, Liu Q, Gong C, Yao Y, Lv X, *et al*. A cytoplasmic NF-κB interacting long noncoding RNA blocks IκB phosphorylation and suppresses breast cancer metastasis. Cancer Cell 2015;27:370-81.
27. Weidle UH, Birzele F, Kollmorgen G, Rüger R. Long non-coding RNAs and their role in metastasis. Cancer Genomics Proteomics 2017;14:143-60.
28. Liang H, Zhang J, Zen K, Zhang CY, Chen X. Nuclear microRNAs and their unconventional role in regulating non-coding RNAs. Protein Cell 2013;4:325-30.
29. Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing MiRNA-LncRNA interactions. Methods Mol Biol 2016;1402:271-86.
30. Rashid F, Shah A, Shan G. Long non-coding RNAs in the cytoplasm. Genomics Proteomics Bioinformatics 2016;14:73-80.
31. Wang DS, Zhong B, Zhang MS, Gao Y. Upregulation of serum miR-103 predicts unfavorable prognosis in patients with colorectal cancer. Eur Rev Med Pharmacol Sci 2018;22:4518-23.
32. Nonaka R, Miyake Y, Hata T, Kagawa Y, Kato T, Osawa H, *et al*. Circulating miR-103 and miR-720 as novel serum biomarkers for patients with colorectal cancer. Int J Oncol 2015;47:1097-102.