Med Oncol (2014) 31:781
DOI 10.1007/s12032-013-0781-7

ORIGINAL PAPER

The expression of miR-25 is increased in colorectal cancer and is associated with patient prognosis

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Received: 7 October 2013 / Accepted: 20 November 2013 / Published online: 29 November 2013

Abstract MicroRNA-25 (miR-25) has recently been found to be involved in many critical processes in human malignancies. We aimed to investigate the expression pattern and prognostic role of miR-25 in colorectal cancer. Colorectal cancer and adjacent normal specimens from 186 patients who had not received neoadjuvant chemotherapy were collected. The expression of miR-25 was detected with a quantitative real-time PCR assay, and the association of miR-25 with overall patient survival was analyzed via statistical analysis. The results indicated that the level of miR-25 expression was significantly elevated in colorectal cancer compared with the level observed in the adjacent normal tissue. It was also demonstrated that miR-25 expression is associated with tumor invasion, lymph node metastasis, distant metastasis and the TNM stage of colorectal cancer. In addition, a Kaplan–Meier analysis revealed that an increased level of miR-25 expression is associated with a poor overall survival of patients. A multivariate survival analysis also indicated that miR-25 is an independent prognostic marker after adjusting for known prognostic factors. These results prove that miR-25 expression is increased in colorectal cancer and is associated with tumor progression. This study also provides the first evidence that miR-25 is an independent prognostic factor for patients with colorectal cancer, indicating the potential role of miR-25 as a highly specific and sensitive biomarker.

Keywords miR-25 · Colorectal cancer · Real-time PCR · Prognosis

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Introduction

Colorectal cancer is the fourth most common cancer in men and the third most common cancer in women worldwide [1]. This cancer was once a malignancy that primarily occurred in longstanding developed countries; however, high colorectal cancer rates have been recently observed in developing countries such as China in which the risk was once low [2, 3]. In the last decade, the incidence and mortality rate of colorectal cancer have been increasing simultaneously in China, where it ranks as the fourth most common malignant tumor [3, 4]. Despite an earlier diagnosis and the progress in surgical, radiotherapy and neoadjuvant chemotherapy treatments, many colorectal cancers remain incurable [5, 6]. Because the prognosis of colorectal cancer is directly correlated with the extent of the local and metastatic tumor spread, the diagnosis and prevention of early tumor metastasis has become one of the most important topics in recent tumor studies.

MicroRNAs (miRNAs) are a class of highly conserved, single-stranded, small noncoding RNA molecules that are known to regulate endogenous gene expression through translational repression and messenger RNA cleavage [7, 8]. miRNAs can inhibit gene expression at the post-transcriptional level by binding to the 3′ untranslated region of a target mRNA, which may result in mRNA degradation or the inhibition of translation depending on the degree of complementary base pairing [9, 10]. It has

been widely accepted that miRNAs play pivotal roles in various biological processes, including development, metabolism, cell proliferation, differentiation and apoptosis [8]. Recently, accumulating evidence has also suggested that miRNAs participate in tumor oncogenesis, including during the processes of angiogenesis, invasion and metastasis [11]. However, some miRNAs were found to act as tumor suppressors, whereas others acted as oncogenes, depending on the targets of the miRNAs, which may provide insights into the functional detection of human malignancies [12]. Recent studies have shown that the expression of miRNAs is deregulated in various human malignancies, such as breast cancer, colon cancer, lung cancer, pancreatic cancer and chronic lymphocytic leukemia [12]. In human colorectal cancer, multiple miRNAs with aberrant expression have been identified. miR-21, miR-31, miR-135, miR-17-92 and miR-196a were found to be overexpressed and to play oncogenic roles, while miR-34, miR-195 and miR-365 were found to be decreased, indicating the tumor-suppressive roles of these miRNAs [13–18]. These results indicate that miRNAs play diverse and crucial roles in human malignancies, including colorectal cancer. Because the expression profile of miRNAs has been shown to be highly tissue specific, demonstrating the functional and clinical significance of a specific miRNA may provide clinically relevant insights into the function of the miRNA and efficacious cancer management. miR-25 is 22 nucleotides in length, is hosted by the minichromosome maintenance protein-7 (MCM7) gene and is transcribed as part of the mir-106b-25 polycistron [19]. Furthermore, PTEN mRNA has been identified as one of the targets of miR-25 [19]. miR-25 expression was found to be increased in a number of human malignancies, such as pediatric brain cancer, medulloblastomas, prostate cancer, hepatocellular cancer, gastric cancer and lung adenocarcinoma [19–23]. However, to our knowledge, the expression pattern and clinical significance of miR-25 in colorectal cancer have not yet been reported.

In the present study, we investigated the expression level of miR-25 in clinical colorectal specimens and adjacent normal tissues and analyzed the association of miR-25 with the overall survival of the patients.

Materials and methods

Patients and specimens

The present study was approved by the Ethics Committee of Shaanxi Provincial People's Hospital. All the members involved provided written informed consent. Fresh clinical colorectal cancer specimens and adjacent normal tissues were collected from 186 patients who underwent surgery between January 2007 and December 2008 in Shaanxi Provincial People's Hospital. None of these patients had received chemotherapy prior to surgery. In addition, normal tissue samples were taken as normal control samples from 18 patients who underwent surgery for reasons other than malignancy. The histomorphology of all the tissue specimens was confirmed by the Department of Pathology, Shaanxi Provincial People's Hospital. After surgical resection, the specimens were put immediately into liquid N_2 for 10 min and then into a −70 °C ultra-freezer for later mRNA isolation. The patients' clinical information, such as age, sex, differentiation status and TNM stage, was collected and stored in a database. A complete follow-up was available for at least 5 years. Overall survival is defined as the time elapsed from the surgery to the death of the patients with colorectal cancer. The follow-up information of all the participants was updated every 3 months by a telephone call and questionnaire letters. The death of the participants was ascertained by a report from the family and verified by the review of public records.

Quantitative real-time PCR

Total RNA was purified from all the 186 colorectal cancer and matching adjacent normal specimens using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). Only those total RNA samples with an OD A260/A280 ratio close to a value of 2.0, which indicates that the RNA is pure, were subsequently analyzed. The miR-25 and RNU44 internal control-specific cDNAs were synthesized from the total RNA using gene-specific primers according to the TaqMan MicroRNA assays protocol (Applied Biosystems, Foster City, CA, USA). The reverse transcription products were then amplified and detected by real-time PCR using a Taqman MicroRNA Assay (Applied Biosystems) specific for hsa-miR-25. Each sample was examined in triplicate, and the raw data are presented as the relative quantification of miR-25 expression evaluated by the comparative cycle threshold (CT) method, normalized with respect to RNU44. The mean normalized miR-25 expression ± the standard deviation (SD) was calculated from triplicate analyses. The real-time PCR was performed with an ABI 7500 system (Applied Biosystems), and the comparative $2^{-\Delta\Delta Ct}$ analysis was performed using SDS 2.2.2 software (Applied Biosystems).

Statistical analysis

The difference in the expression of miR-25 between colorectal cancer and the normal specimen was analyzed with a Student's *t* test. Associations between miR-25 expression and the clinicopathological characteristics were analyzed via a Mann–Whitney test or a Kruskal–Wallis test, as appropriate. The survival curves were estimated

using the Kaplan–Meier method, and differences in the survival distributions were evaluated with a logrank test. A Cox proportional hazards modeling of the factors potentially related to survival was performed to identify those factors that might have a significant influence on survival. Differences with a P value of 0.05 or less were considered to be statistically significant.

Results

miR-25 expression detected in colorectal cancer

In a real-time PCR assay, 186 cases of colorectal cancer, matching adjacent normal specimens and 18 cases of normal control specimens were investigated. The relative expression of miR-25 normalized to RNU44 in colorectal cancer was found to be 7.68 ± 1.56 (mean ± SD), while that in matching adjacent normal and normal control specimens was 2.85 ± 0.58 and 1.61 ± 0.32, respectively. The results of the statistical analysis proved that the expression of miR-25 in colorectal cancer was significantly higher than that in the adjacent and normal control specimens ($P < 0.05$). These results indicated that miR-25 might play an oncogenic role in colorectal cancer. To facilitate further analysis of the association between miR-25 expression and clinical features, we manually defined colorectal cancer cases with a level of miR-25 expression lower than 3.43 (mean + SD for the expression of miR-25 in adjacent specimens) as a low miR-25 expression group, while specimens with a level of miR-25 expression no lower than 3.43 were defined as a high miR-25 expression group. Thus, among the 186 cases of colorectal cancer, 78 cases were assigned to the low expression group, while 108 cases were assigned to the high expression group.

Table 1 Association of miR-25 with clinicopathological characteristics

Variable	n	miR-25 expression		P
		Low	High	
Total	186	78	108	
Gender				0.946*
Male	102	43	59	
Female	84	35	49	
Age				0.793*
< 60	110	47	63	
≥ 60	76	31	45	
Differentiation				0.808†
Poor	62	24	38	
Moderate	86	37	49	
Well	38	17	21	
Invasion				<0.001*
T1 + T2	90	54	36	
T3 + T4	96	24	72	
Lymph node metastasis				<0.001*
Negative	76	50	26	
Positive	110	28	82	
Distant metastasis				0.001*
Negative	164	76	88	
Positive	22	2	20	
TNM stage				<0.001†
I	24	18	6	
II	52	22	20	
III	88	26	62	
IV	22	2	20	

* P value when expression levels were compared using Mann–Whitney test

† P value when expression levels were compared using Kruskal–Wallis test

Relationship between miR-25 and clinicopathological characteristics of colorectal cancer

Our investigation revealed that miR-25 expression is increased in colorectal cancer, which indicates that miR-25 might play an oncogenic role. Therefore, we further investigated the association of miR-25 expression with the clinicopathological characteristics of the patients to explore the potential role of miR-25 in colorectal cancer progression. The statistical analysis results, shown in Table 1, indicate that increased miR-25 expression is associated with advanced colorectal cancer invasion because high levels of miR-25 expression were more frequently detected in T3 and T4 tumors ($P < 0.001$). Regarding tumor metastasis, elevated levels of miR-25 expression were related to positive lymph node and distant metastasis because high miR-25 expression was detected more frequently in tumors with lymph node ($P < 0.001$) or distant metastasis ($P = 0.001$). In addition, when we measured the patients' clinical features according to the TNM staging system, we found that miR-25 expression was significantly associated with the TNM stage of the colorectal cancer because a high level of miR-25 expression was more likely to be detected in tumors with an advanced TNM stage ($P < 0.001$). These results suggest that miR-25 might play an oncogenic role in the progression of colorectal cancer. However, high levels of miR-25 expression were not found to be associated with the patients' gender ($P = 0.946$), age ($P = 0.793$) or differentiation status ($P = 0.808$).

The relationship between miR-25 and the overall survival of patients with colorectal cancer

During the entire follow-up period, 94 of the 186 patients (50.5 %) with colorectal cancer died, and the median

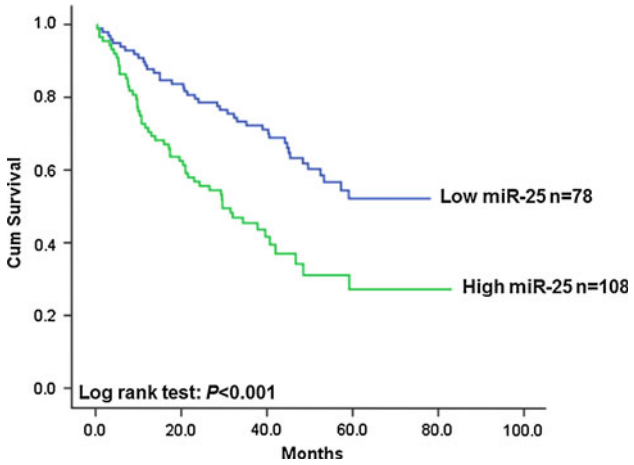

Fig. 1 Kaplan–Meier postoperative survival curve for patients with colorectal cancer and miR-25 expression

overall survival time of all the recruited patients was 48 months. A Kaplan–Meier analysis was applied to examine the prognostic value of miR-25 expression regarding the overall survival of the patients. The results indicated that the patients who had colorectal cancer with a high level of miR-25 expression had worse overall survival rates compared with those who had cancers with low miR-25 expression (logrank test: $P < 0.001$, Fig. 1). The median survival time of patients with a low level of miR-25 expression could not be estimated because more than 50 % of the patients survived during the follow-up period, while the postoperative median survival time of the patients with high levels of miR-25 expression was 30 months (95 % CI 17.7–41.5). When the unadjusted hazard ratio (HR) was considered with a low level of miR-25 expression as the reference (1.00), the patients with colorectal cancer with a high level of miR-25 expression had a 2.29-fold higher risk of death (95 % CI 1.51–3.47; $P < 0.001$). Regarding the clinicopathological characteristics, lymph node metastasis (logrank test: $P = 0.011$), distant metastasis (logrank test: $P < 0.001$) and TNM stage (logrank test: $P < 0.001$) were also shown to be associated with overall survival because patients who had colorectal cancer with lymph node metastasis, distant metastasis or an advanced grade had worse overall survival rates and a higher risk of death. However, sex (logrank test: $P = 0.596$) or age (logrank test: $P = 0.827$) had no prognostic value regarding the overall survival of patients with colorectal cancer.

Because miR-25 expression was found to be associated with the overall survival of patients in the univariate survival analysis, we further investigated whether miR-25 could serve as an independent prognostic marker for patients with colorectal cancer. Because lymph node and distant metastasis information was included in the TNM stage data, we used a Cox proportional hazards model

Table 2 Association of miR-25 expression and clinical factors with overall survival of patients with colorectal cancer

	Unadjusted HR[a] (95 % CI)	*P*	Adjusted HR[b] (95 % CI)	*P*
miR-25				
Low expression	–		–	
High expression	2.29 (1.51–3.47)	<0.001	1.90 (1.22–2.97)	0.005
Gender				
Female	–		–	
Male	1.16 (0.68–1.98)	0.596	1.28 (0.69–2.36)	0.429
Age				
< 60	–		–	
≥ 60	1.07 (0.69–1.68)	0.760	1.06 (0.69–1.16)	0.801
TNM stage				
I	–		–	
II	1.59 (0.69–3.68)	0.274	1.77 (0.75–4.16)	0.193
III	3.68 (1.62–8.39)	0.002	3.88 (1.69–8.89)	0.001
IV	8.75 (4.09–18.71)	<0.001	9.10 (4.25–19.49)	<0.001

[a] Hazard ratios in univariate models

[b] Hazard ratios in multivariable models

HR hazard ratio, *95 % CI* 95 % confidence interval

adjusted for sex, age and TNM stage. The results indicated that the adjusted HR of the high miR-25 expression group was 1.90 (95 % CI 1.22–2.97; $P = 0.005$). These results prove that miR-25 is an independent prognostic factor of overall survival for patients with colorectal cancer. Thus, increased miR-25 expression could be an indicator of poor overall survival without consideration of age, sex or TNM stage (Table 2).

Discussion

According to recent studies, more than 1,900 human miRNAs have been identified to date, which are estimated to regulate over 60 % of the genes in mammals [24]. Due to their great importance in the regulation of gene expression, it has been widely accepted that miRNAs are involved in multiple cellular functions, including proliferation, apoptosis and differentiation, and thus have been implicated in diverse physiological and pathological processes ranging from organismal development to cancer [25–27]. Because individual miRNAs in different cancer types have a large number of different gene targets that may result in different functions, the discovery of the function of an individual miRNA may enable deeper insight into the regulation of gene expression and the complexity of cancer progression.

In the present study, we investigated miR-25 expression with a real-time PCR assay in 186 specimens of colorectal

cancer from patients who had not received neoadjuvant chemotherapy. Based on the calculation of relative expression, we analyzed the association of miR-25 with the clinicopathological characteristics as well as the prognosis of the patients. The results indicated that the level of miR-25 expression was increased in colorectal cancer compared with the levels observed in normal tissues, which is consistent with previous investigations focused on other human malignancies showing that miR-25 expression was increased in these tumors. A recent study revealed that when rats were exposed to tamoxifen, a known inducer of liver cancer, hepatic miR-25 expression was increased, which also suggests the participation of miR-25 in carcinogenesis [28]. It was also reported that the inhibition of miR-25 with an inhibitor caused significant suppression of proliferation and a significant increase in cancer cell apoptosis in ovarian cancer, tongue squamous cell carcinoma and gastric cancer [29–31]. These findings demonstrate that the dysregulation of miR-25 may participate in human malignancy and carcinogenesis, including colorectal cancer.

In addition, the present study also proved that miR-25 expression is closely related to colorectal cancer invasion, lymph node metastasis, distant metastasis and the TNM stage. The results revealed that a high level of miR-25 expression was more frequently detected in tumors with deep invasion, lymph node metastasis, distant metastasis or an advanced TNM stage, suggesting the possible participation of miR-25 in colorectal cancer invasion and metastasis. This association is consistent with a previous study of human gastric cancer, which found that miR-25 could remarkably enhance cell proliferation, migration and invasion, whereas the inhibition of miR-25 could significantly decrease the migration and invasion of gastric cancer cells [29]. Based on the present results, together with the evidence above, it is thus proposed that miR-25 may play an important role in colorectal cancer carcinogenesis and progression.

Because miR-25 expression was found to be associated with colorectal cancer invasion and metastasis and considering that cancer invasion and metastasis are crucial factors affecting the prognosis of patients, miR-25 might be a potential prognostic marker for patients with colorectal cancer. To investigate the prognostic role of miR-25 in colorectal cancer, we performed a Kaplan–Meier analysis. The results revealed that the patients who had colorectal cancer with a high level of miR-25 expression had worse overall survival compared to the patients whose tumors had low levels of miR-25 expression, which suggests that miR-25 expression is a prognostic marker for patients with colorectal cancer. To further evaluate the prognostic value of miR-25 in colorectal cancer, we used a Cox proportional hazards model that was adjusted for gender, age and the TNM stage of the patients. The results proved that increased miR-25 expression is a marker of poor overall survival independent of the adjusted factors; therefore, miR-25 could be utilized to determine patients' prognosis without considering the TNM stage. These results indicate that miR-25 could constitute a molecular prognostic marker in conjunction with the TNM stage for patients with colorectal cancer, identifying high risk individuals who are good candidates to receive treatment that is more aggressive. The present results are consistent with a previous investigation on acute leukemia, which found that miR-25 expression was significantly correlated with the overall survival of patients [32]. This positive linkage between miR-25 dysregulation and colorectal cancer carcinogenesis, progression and prognosis may be at least partly caused by the targets of miR-25, such as PTEN, RECK, Bim, Bax, CDH1 and caspase-3 [30, 33]. These results suggest that miR-25 may be used not only for identifying colorectal cancer patients with a higher risk of early tumor relapse but also for providing valuable clues to understanding the possible mechanism of colorectal cancer invasion and metastasis.

In conclusion, we have demonstrated that miR-25 expression is increased in colorectal cancer and is associated with tumor progression. The present study also demonstrated for the first time that miR-25 expression is an independent prognostic factor for patients with colorectal cancer. Therefore, miR-25 may play an important role in the invasion and metastasis of colorectal cancer. It is also possible that miR-25 may serve as a prognostic marker in clinical practice, and the inhibition of miR-25 by specific inhibitors may even become a new therapeutic method for the treatment of colorectal cancer.

Conflict of interests None.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics 2012. CA Cancer J Clin. 2012;62(1):10–29. doi:10.3322/caac.20138.
2. Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. CA Cancer J Clin. 2009;59(6):366–78. doi:10.3322/caac.20038.
3. Deng SX, An W, Gao J, Yin J, Cai QC, Yang M, et al. Factors influencing diagnosis of colorectal cancer: a hospital-based survey in China. J Dig Dis. 2012;13(10):517–24. doi:10.1111/j.1751-2980.2012.00626.x.
4. Chen HM, Weng YR, Jiang B, Sheng JQ, Zheng P, Yu CG, et al. Epidemiological study of colorectal adenoma and cancer in symptomatic patients in China between 1990 and 2009. J Dig Dis. 2011;12(5):371–8. doi:10.1111/j.1751-2980.2011.00531.x.
5. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin. 2012;62(4):220–41. doi:10.3322/caac.21149.
6. Wolpin BM, Meyerhardt JA, Mamon HJ, Mayer RJ. Adjuvant treatment of colorectal cancer. CA Cancer J Clin. 2007;57(3):168–85.

7. Shukla GC, Singh J, Barik S. MicroRNAs: processing, maturation, target recognition and regulatory functions. Mol Cell Pharmacol. 2011;3(3):83–92.
8. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215–33. doi:10.1016/j.cell.2009.01.002.
9. John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA targets. PLoS Biol. 2004;2(11):e363. doi:10.1371/journal.pbio.0020363.
10. Kiriakidou M, Nelson PT, Kouranov A, Fitziev P, Bouyioukos C, Mourelatos Z, et al. A combined computational-experimental approach predicts human microRNA targets. Genes Dev. 2004;18(10):1165–78. doi:10.1101/gad.1184704.
11. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006;6(11):857–66. doi:10.1038/nrc1997.
12. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA. 2006;103(7):2257–61. doi:10.1073/pnas.0510565103.
13. Schee K, Boye K, Abrahamsen TW, Fodstad O, Flatmark K. Clinical relevance of microRNA miR-21, miR-31, miR-92a, miR-101, miR-106a and miR-145 in colorectal cancer. BMC Cancer. 2012;12:505. doi:10.1186/1471-2407-12-505.
14. Schimanski CC, Frerichs K, Rahman F, Berger M, Lang H, Galle PR, et al. High miR-196a levels promote the oncogenic phenotype of colorectal cancer cells. World J Gastroenterol WJG. 2009;15(17):2089–96.
15. Bandres E, Cubedo E, Agirre X, Malumbres R, Zarate R, Ramirez N, et al. Identification by real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Mol Cancer. 2006;5:29. doi:10.1186/1476-4598-5-29.
16. Motoyama K, Inoue H, Takatsuno Y, Tanaka F, Mimori K, Uetake H, et al. Over- and under-expressed microRNAs in human colorectal cancer. Int J Oncol. 2009;34(4):1069–75.
17. Nie J, Liu L, Zheng W, Chen L, Wu X, Xu Y, et al. microRNA-365, down-regulated in colon cancer, inhibits cell cycle progression and promotes apoptosis of colon cancer cells by probably targeting Cyclin D1 and Bcl-2. Carcinogenesis. 2012;33(1):220–5. doi:10.1093/carcin/bgr245.
18. Liu L, Chen L, Xu Y, Li R, Du X. microRNA-195 promotes apoptosis and suppresses tumorigenicity of human colorectal cancer cells. Biochem Biophys Res Commun. 2010;400(2):236–40. doi:10.1016/j.bbrc.2010.08.046.
19. Poliseno L, Salmena L, Riccardi L, Fornari A, Song MS, Hobbs RM, et al. Identification of the miR-106b ~25 microRNA cluster as a proto-oncogenic PTEN-targeting intron that cooperates with its host gene MCM7 in transformation. Sci Signal. 2010;3(117):ra29. doi:10.1126/scisignal.2000594.
20. Birks DK, Barton VN, Donson AM, Handler MH, Vibhakar R, Foreman NK. Survey of MicroRNA expression in pediatric brain tumors. Pediatr Blood Cancer. 2011;56(2):211–6. doi:10.1002/pbc.22723.
21. Petrocca F, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, et al. E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. Cancer Cell. 2008;13(3):272–86. doi:10.1016/j.ccr.2008.02.013.
22. Dacic S, Kelly L, Shuai Y, Nikiforova MN. miRNA expression profiling of lung adenocarcinomas: correlation with mutational status. Mod Pathol. 2010;23(12):1577–82. doi:10.1038/modpathol.2010.152.
23. Li Y, Tan W, Neo TW, Aung MO, Wasser S, Lim SG, et al. Role of the miR-106b-25 microRNA cluster in hepatocellular carcinoma. Cancer Sci. 2009;100(7):1234–42. doi:10.1111/j.1349-7006.2009.01164.x.
24. Esteller M. Non-coding RNAs in human disease. Nat Rev Genet. 2011;12(12):861–74. doi:10.1038/nrg3074.
25. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet. 2004;5(7):522–31. doi:10.1038/nrg1379.
26. Mendell JT. MicroRNAs: critical regulators of development, cellular physiology and malignancy. Cell Cycle. 2005;4(9):1179–84.
27. Kasinski AL, Slack FJ. Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. Nat Rev Cancer. 2011;11(12):849–64. doi:10.1038/nrc3166.
28. Pogribny IP, Tryndyak VP, Boyko A, Rodriguez-Juarez R, Beland FA, Kovalchuk O. Induction of microRNAome deregulation in rat liver by long-term tamoxifen exposure. Mutat Res. 2007;619(1–2):30–7. doi:10.1016/j.mrfmmm.2006.12.006.
29. Zhao H, Wang Y, Yang L, Jiang R, Li W. miR-25 promotes gastric cancer cells growth and motility by targeting RECK. Mol Cell Biochem. 2013;. doi:10.1007/s11010-013-1829-x.
30. Zhang H, Zuo Z, Lu X, Wang L, Wang H, Zhu Z. MiR-25 regulates apoptosis by targeting Bim in human ovarian cancer. Oncol Rep. 2012;27(2):594–8. doi:10.3892/or 2011.1530.
31. Xu JY, Yang LL, Ma C, Huang YL, Zhu GX, Chen QL. MiR-25-3p attenuates the proliferation of tongue squamous cell carcinoma cell line Tca8113. Asian Pac J Trop Biomed. 2013;6(9):743–7. doi:10.1016/S1995-7645(13)60130-3.
32. Wang Y, Li Z, He C, Wang D, Yuan X, Chen J, et al. MicroRNAs expression signatures are associated with lineage and survival in acute leukemias. Blood Cells Mol Dis. 2010;44(3):191–7. doi:10.1016/j.bcmd.2009.12.010.
33. Xu X, Chen Z, Zhao X, Wang J, Ding D, Wang Z, et al. MicroRNA-25 promotes cell migration and invasion in esophageal squamous cell carcinoma. Biochem Biophys Res Commun. 2012;421(4):640–5. doi:10.1016/j.bbrc.2012.03.048.