IBBJ
Winter 2016, Vol 2, No 1

Original Article

Expression Analysis of Let-7a miRNA and its Target Gene NRAS in Cytogenetically Normal Family with Myelodysplastic Syndrome

Shermineh Heydari[1], Elaheh Ghods[1], Majid Mojarrad[1], Zahra Mozaheb[2], Ehsan Ghayoor Karimiani[3], Mohammad Hassanzadeh Nazarabadi[1*]

1. Medical genetic department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

2. Hematology-Oncology department, Mashhad University of Medical Sciences, Mashhad, Iran.

3. *Molecular Diagnostic unit, Research and Education department, Razavi Hospital, Mashhad, Iran.*

Submitted 13 Jul 2016; Accepted 17 Aug 2016; Published 19 Sep 2016

Myelodysplastic syndrome (MDS), is a group of heterogeneous disorders of hematopoietic stem cell colonies which is determined by incomplete hematopoiesis in one or more cell lines. The incidence increases with age and it has less been reported among patients under 50 years of age. The commonest form of MDS is sporadic, and familial occurrence of MDS is rare. Patients with familial MDS are younger at the time of diagnosis than individuals with sporadic disease and are faced with an unusual family history of more than one first-degree relative with MDS. In this study, we investigated a family with 16 members which has a family history of MDS in three consecutive generations. Cytogenetic and chromosome analyzes were performed on the 16 members of the family. Moreover, let-7a miRNA expression and its target gene, NRAS were evaluated compared to the control group. The karyotype analysis in this family shows two patients with abnormal karyotype, whereas expression of the let-7a and NRAS in this family showed a significant decrease compared to the control group. Regarding the decreased expression of let-7a in patient samples, this miRNA can be further considered as a marker for early diagnosis of MDS disease.

Keywords: Myelodysplastic syndrome (MDS), familial MDS, cytogenetic, *let-7a*, *NRAS*

Myelodysplastic syndrome (MDS) is a group of heterogeneous disorders of hematopoietic stem cell (HSC) colonies which is determined by incomplete hematopoiesis in one or more cell lines and defect in the maturation of myelopoiesis progenitor cell and it causes peripheral blood cytopenia, morphological dysplasia, progressive bone marrow failure and an increased risk of transforming to acute myeloid leukemia (AML) (1-3). The incidence of this disease increases with age, and its prevalence is mainly in adults and it has less been reported among patients under 50 years of age (4, 5).

The commonest form of MDS is sporadic and the disease occurs most frequently in older people with a median age of 65 years (6). Familial

*Correspondence: Medical genetic department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
E-mail: nazarabadim@mums.ac.ir

[Downloaded from ibbj.org on 2026-09-15]

occurrence of MDS is rare, but can provide a valuable resource for research. To date, fewer families have been examined for this disease. These investigations have been difficult due to the rarity of familial MDS and in most cases pedigrees are too small and also the access to the entire family is very difficult. Patients with familial MDS are younger at the time of diagnosis than individuals with sporadic disease and are faced with an unusual family history of more than one first-degree relative with MDS. Most of the pedigrees which were reported so far, demonstrated a dominant autosomal pattern of inheritance (7, 8).

Diagnosis, prognosis and classification of MDS is of great importance for physicians and is challenging in subjects affected with MDS (9). Cytogenetic abnormalities can be seen in 50% of patients with MDS and the rest are patients with a normal karyotype (10, 11). Therefore, for the early and accurate diagnosis of this disease, new markers are needed. Micro RNAs (miRNAs) play a crucial role in the regulation of the hematopoietic system and are involved in many of the specialized processes of the hematopoietic cells (12). miRNAs, are small non-coding RNAs, with 18-25 nucleotides length which are responsible for the regulation of gene expression at post transcriptional level (13). The profiles and expression of many miRNAs are investigated in tumor tissues compared to normal ones to achieve a panel of markers for the diagnosis and prognosis of cancers and their classification (14, 15).

Let-7 miRNA family is one of the first miRNAs which were identified and have been highly conserved during evolution (16). In humans, different genes of *let-7* have been reported that are located in regions of chromosomes which are deleted in human cancers. Many studies have shown that miRNAs regulate *RAS* which acts as a crucial oncogene in humans. Three human RAS genes have *let-7* binding sites in their 3 'UTR through which the cell cycle can be regulated (17).

The investigation of miRNAs expression in patients with MDS can be used as an early diagnosis tool for prognosis and classification of this disease. Molecular study and cytogenetic characteristics of familial MDS, such as cDNA expression profiles between affected and unaffected family members help to reveal the molecular pathways that are involved in this disease (18).

Materials & methods

Patients

Peripheral blood samples from 16 members of a family with MDS (Figure 1) and 34 healthy normal control groups aged between 10-70 years old were

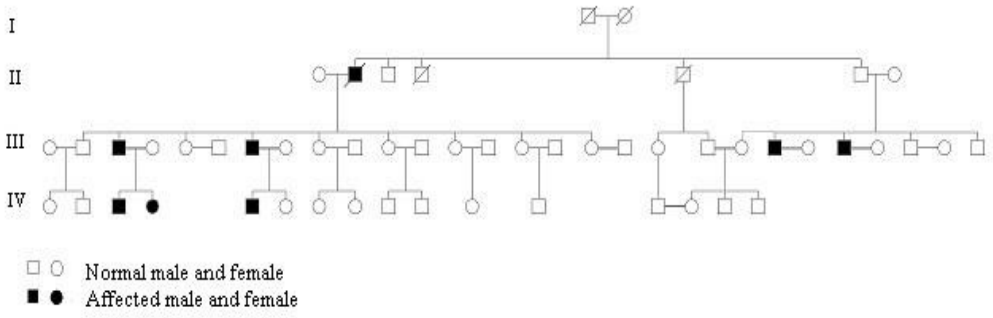

Figure 1. Pedigree of the family presenting MDS.

[Downloaded from ibbj.org on 2026-09-15]

collected. Since the incidence of this disease increases with age, suitable control samples were chosen proportionate to family members' age. Blood cells counting was performed for all normal control group subjects and they were screened in terms of being free from MDS disease. Among the family members, 6 patients had MDS and the rest of them were healthy. Sampling was conducted from two generations of this family. In the first generation of the affected family, the father died at the age of 50 due to AML which was the consequence of MDS and unfortunately we did not have access to his blood sample. According to world health organization (WHO) criteria, all patients of this family were in refractory cytopenia with unilineage dysplasia (RCUD) group, and according to revised international prognostic scoring system (IPSS-R) for MDS, they were in the low risk group with the average survival time of 3.5 years. Written informed consent was obtained from all participants. Moreover, this research project has been approved by the ethics committee of Mashhad University of Medical Sciences.

Cytogenetic analyzes

Chromosomal analysis was performed on the basis of GTG-banding technique at high resolution. Peripheral blood lymphocytes were cultured in RPMI 1640 medium (Gibco®) enriched with FBS phytohemagglutinin and L-glutamine. The cells were cultured for 72 h at 37 °C in a CO_2 incubator. Cultures were stopped by adding colcemid solution 2 h before harvesting, then the cells were exposed to hypotonic solution and fixed. Metaphase chromosome spread was prepared and G-banding technique was applied with the use of trypsin-giemsa (GTG). Analysis of the karyotype was performed in at least 20 metaphase cells. Karyotypes were described according to the international system of human cytogenetic nomenclature (ISCN).

Molecular analyzes

Peripheral blood mononuclear cells were isolated by ficoll technique, then RNA and miRNA were extracted separately by using Hybriyd-R miRNA kit (Gene ALL company) and then cDNA synthesis was performed using miScript II RT Kit (Qiagen company). In order to study *NRAS* gene expression and *let-7a*, in the next stage quantitative real-time PCR reactions were performed using Syber Green and in triplicate for each reaction using Step one (ABI company). *GAPDH* and *mir-192* were used as internal control for *NRAS* and *let-7a* genes, respectively. Primers of *NRAS* and *GAPDH* genes were designed using the online primer 3 software. To investigate the microRNA expression Qiagen company primers were used. To confirm the sequencing of amplified fragment, the products of real time PCR were cloned and sequenced using E.coli bacteria and pBluescript SK (+) vector.

Statistical analyzes

Fold Change, related to *NRAS* and *let-7a* miRNA genes were calculated in all patients compared to control group using the equation $2^{-\Delta\Delta CT} = 2^{-(\Delta CT \text{ patient} - \Delta CT \text{ normal})}$. Independent T test was conducted using SPSS statistical software to compare the overall situation of fold change.

Results

Cytogenetic analyzes

Out of 16 members of the studied family, 14 revealed a normal karyotype whereas the two other members appeared with abnormal karyotype involving a partial trisomy in 1p34 region in one subject and a robertsonian translocation between chromosomes number 13 and 14 in the other subject

Real-time PCR reaction

The results of T-independent test for the expression (fold change) obtained from the equation

[Downloaded from ibbj.org on 2026-09-15]

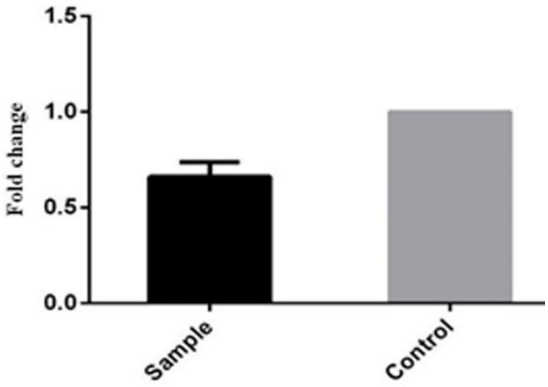

Figure 2. Comparison of *let-7a* expression in MDS family and control group. The expression of *let-7a* was higher in normal subjects (control) compared to MDS family members (sample); P< 0.0001.

$2^{-\Delta\Delta CT}$ for *let-7a* miR in family compared to the control group, showed a P value less than 0.0001, indicating a significant decrease in expression of *let-7a* miR compared to the control group (Figure 2).

Comparison of *let-7a* miRNA expression in samples of women and men in the family with the control group showed reduced expression of *let-7a* miRNA (Figure 3).

T-independent test results for the investigation of the expression obtained from the equation $2^{-\Delta\Delta CT}$ for *NRAS* gene in the family compared to the control group show P value less than 0.0001 which shows significant decrease of *NRAS* gene expression compared to the control group (Figure 4).

T test results in women of the family compared to women in the control group did not show

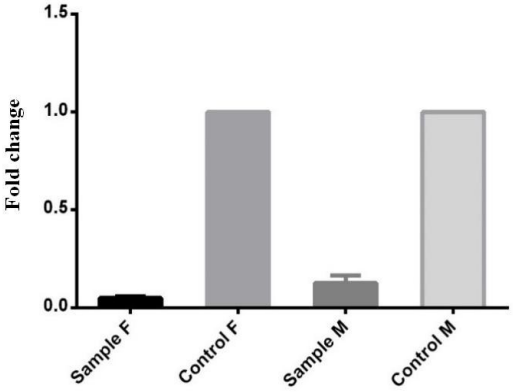

Figure 3. Comparison of *let-7a* in women and men of MDS family and control group.*Let -7a* expression was significantly decreased in both male (Sample M) and female (Sample F) subjects of MDS family in comparison with healthy male (Control M) and female (Control F) subjects; P< 0.0001.

significant difference (P value = 0.5899) and in men of the family compared to men in control showed reduced expression of *NRAS* in patients with P value less than 0.0001 (Figure 5).

Discussion

MDS is a disorder of HSC colonies which is determined by defect in the maturation of myelopoietic progenitor cells (19). The familial cases are rare (20) and few studies have so far been conducted on the disease inheritance and contributing factors in the disease creation. Examination of the families suffering from MDS may assist unde-

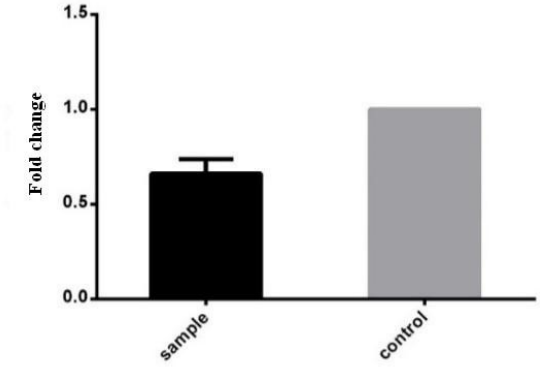

Figure 4. Comparison of *NRAS* expression in MDS family and control group. The expression of *NRAS* was higher in normal subjects (control) compared to MDS family members (sample); P< 0.0001.

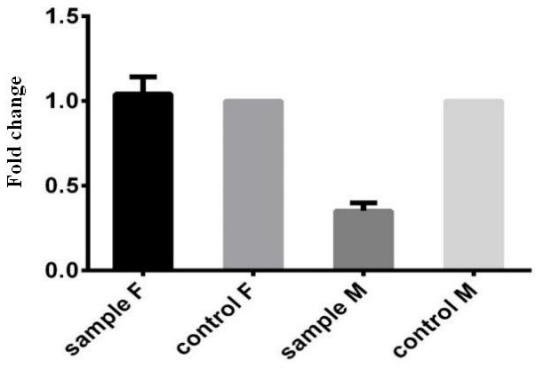

Figure 5. Comparison of *NRAS* in women and men of MDS family and control group. *NRAS* expression was significantly decreased in male (sample M) subjects of MDS family in comparison with healthy male (control M) subjects (P< 0.0001), but its expression did not vary significantly between female MDS (sample F) and normal female (control F) subjects.

[Downloaded from ibbj.org on 2026-09-15]

rstanding of the disease mechanism.

In the present study, cytogenetic alterations, miRNA *let-7a* expression and the possible target gene, *NRAS*, have been investigated in a MDS affected family. In a similar study, three generations of the family with AML or MDS members have been examined (21).

In the present study,14 members of the family had normal karyotype. One member showed 13q14q robertsonian translocation the other member showed partial trisomy in 1p34 region. No studies has yet been reported on 13q14q robertsonian translocation along with MDS disease. The anomaly seems an accidental finding in the reported family; however, the affected member was informed and the necessary preventaional instructions were provided. In addition, information on possible childbirth with Patau syndrome was given to the family. The second case showed partial trisomy in 1p34 region. This might be a *de novo* chromosomal defect due to the disease regarding the lack of report on other family members.

Retrospective studies have disclosed that cytogenetic abnormality have been observed in only 50 percent of MDS patients (22). Hence, we need newer markers to detect genetically the disease in early stages, and accurately. Investigation of families with both established and newer molecular genetic techniques may also identify novel causative mechanisms. In addition, expression profile comparison between MDS familial and sporadic cases can reveal the differences and similarities in paths which migth be deregulated (7).

Many miRNAs deregulation have been proved in different diseases (16, 23). Human cancer profiles analysis has proved that miRNAs will be deregulated noticeably in these cancers. Most of the time they lead to cancer when cells grow abnormally and lose apoptosis function. Recent studies has showed that miRNAs control cellular growth and apoptosis (24). Detection of miRNAs which are expressed differently in tumor and normal samples may assist in identification of miRNAs involved in human cancers and their expression profile may be a useful biomarker for cancer detection (25). Candidate miRNAs expressional decrease or increase analysis is a proper approach for miRNAs function study in cancers (26).

On the other hand, decrease in miRNA *let-7a* expression has been observed showng its role as a tumor suppressor in cancers (27). Precious information exists on miRNAs expressional alterations mechanism in cancers but little information has been reported on their alterations in MDS.

Some abnormal miRNAs expression in bone marrow cause defection in HSC self-renewal and transforming to AML (19). In addition, miRNA expressional alterations inside MDS mature blood cells may cause defect in differentiation which causes neutropenia, thrombocytopenia and anemia. miRNAs expressional markers in normal CD34+ cells and AML cell has revealed some key miRNAs in AML pathobiology (28). Also, some evidence has been obtained on miRNA expressional alterations due to cytogenetic alterations. These alterations relationship has been proved in leukaemia with cytogenetic profile (29, 30).

In the present study, no relationship has been observed between cytogenetic alterations and targeted miRNA expression. In comparison with another study conducted by Hussein et al. who reported a significant relationship between few miRNAs expression alterations and cytogenetic alterations and detected miRNA markers among patients with normal and abnormal karyotype (31). Different performance approaches, targeted miRNAs type, sample size and familial/sporadic MDS type migth be at the origin of differences observed with our findings. Also, in another study

[Downloaded from ibbj.org on 2026-09-15]

carried out by the above-said group, it was revealed that mir-150 presents a noticeable increase in expression in MDS patients with 5q deletion (32).

Many studies have shown that miRNA regulates *RAS* which acts as a crucial oncogene in humans. Three human *RAS* genes have *let-7a* binding sites in their 3' UTR that expose these oncogenes to the *let-7a* through which they may regulate the cell cycle (17).

In the present study, the expression of *let-7a* miRNA decreased significantly (P value< 0.0001) between the control group and members of the family (affected and healthy). So far, no studies have been conducted on the expression of the miRNA in the peripheral blood mononuclear cells of patients with MDS. The only similar study was conducted by Zuo et al., in which *let-7a* miRNA expression was evaluated in the plasma of patients with MDS with findings in line with the results that we have achieved (33).

The expression of *let-7a* miRNA has been studied in other cancers. Takamizawa et al. showed that the expression level of *let-7a* was decreased *in vitro* and *in vivo*. They also showed that the increase of miRNA expression in A549 lung adenocarcinoma cell line, prevents cells growth (34). Moreover, in another study, Johnson et al. showed that lung tumor tissue has significant reduction in the expression level of *let-7a*. The RAS protein levels increased significantly in lung tumor patients which suggest that *let-7a* acts as a regulator of *RAS* in the oncogenic pathway in lung cancer (35).

In the present study the expression of *NRAS* was decreased in the studied family members in comparison with the control group, which was contrary to our expectation as *NRAS* is a *let-7a* target, which decreased among our studied family members, suggesting a tumor suppressor function for this miRNA. Our observation is opposed to the results reported by Johnson et al. on lung cancer (35). This controversy may reflect differences in the mechanism of action of this miRNA in two different tissues.

On the other hand, it seems that *let-7a* miRNA does not affect *NRAS* in bone marrow cells. In addition *let-7a* miRNA has other targets which may be deregulatrd by the decreased expression of this miRNA. Each of these targets can potentially be involved in MDS development through different mechanisms, such as cell proliferation increase, apoptosis and cell cycle deregulation (36).

Furthermore, studies have found that *RAS* has a dual nature in apoptosis, acting as both pro- or anti-apoptotic agent depending on the downstream pathway that is activated by *RAS*. Therefore, the decreased expression of *RAS* in the studied family may have acted in a way that leaded to the loss of RAS apoptotic function and activated the anti-apoptotic mechanism in cells carrying the disease (37) .

The decrease in expression of *let-7a* miRNA and its target gene, *NRAS* in healthy individuals of the family compared to the normal population may indicate a genetic predisposition in these people for the development of MDS disease. Results of this study propose the necessity of carrying a functional study to prove the role of *NRAS* and *let-7a* miRNA in MDS disease.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgement

We would like to express our appreciations to the vice chancellor for research of Mashhad University of Medical Sciences for providing the grant of this project.

References

1. Gill H, Leung A Y, Kwong Y-L. Molecular and Cellular Mechanisms of Myelodysplastic

[Downloaded from ibbj.org on 2026-09-15]

Syndrome: Implications on Targeted Therapy. Int J Mol Sci. 2016;17:440.

2. Avgerinou C, Alamanos Y, Zikos P, et al. The incidence of myelodysplastic syndromes in Western Greece is increasing. Ann Hematol. 2013;92:877-87.

3. Neukirchen J, Schoonen W M, Strupp C, et al. Incidence and prevalence of myelodysplastic syndromes: data from the Düsseldorf MDS-registry. Leuk Res. 2011;35:1591-6

4. Ma X. Epidemiology of myelodysplastic syndromes. Am J Med. 2012;125:S2-S5.

5. Sekeres M A. The epidemiology of myelodysplastic syndromes. Hematol Oncol Clin North Am. 2010;24:287-94.

6. Rollison D E ,Howlader N, Smith M T, et al. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. Blood. 2008;112:45-52.

7. Liew E, Owen C. Familial myelodysplastic syndromes: a review of the literature. Haematologica. 2011;96:1536-42.

8. Przychodzen B P, Makishima H, Dienes B, et al. Hereditary mutations are present as pathogenic or predisposition factors in otherwise typical adult MDS. Blood. 2014;124:3233.

9. Bejar R. Clinical and genetic predictors of prognosis in myelodysplastic syndromes. Haematologica. 2014;99:956-64.

10. Bacher U, Schanz J, Braulke F, et al. Rare Cytogenetic Abnormalities in Myelodysplastic Syndromes. Mediterr J Hematol Infect Dis. 2015;7.

11. Haase D. Cytogenetic features in myelodysplastic syndromes. Ann Hematol. 2008;87:515-26.

12. Pons A, Nomdedeu B, Navarro A, et al. Hematopoiesis-related microRNA expression in myelodysplastic syndromes. Leuk Lymphoma. 2009;50:1854-9.

13. Melo S A, Kalluri R. Molecular pathways: microRNAs as cancer therapeutics. Clin Cancer Res. 2012;18:4234-9.

14. Hayes J, Peruzzi P P, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med. 2014;20:460-9.

15. Acunzo M, Romano G, Wernicke D, et al. MicroRNA and cancer–a brief overview. Adv Biol Regul. 2015;57:1-9.

16. Almeida M I, Reis R M, Calin G A. MicroRNA history: discovery, recent applications, and next frontiers. Mutat Res. 2011;717:1-8.

17. Guan J, Guo S, Liu M. Let-7 Family miRNAs Represent Potential Broad-Spectrum Therapeutic Molecules for Human Cancer. J Genet Syndr Gene Ther. 2015;6:1.

18. Iverson N, Galili N, Ali A M, et al. Current View of miRNA with Tumor Suppressor Function, Exploring MDS and AML as Models. Signal Transduction Insights. 2014;3:1-10.

19. Deeg H J, Scott B L, Fang M, et al. Five-group cytogenetic risk classification, monosomal karyotype and outcome after hematopoietic cell transplantation for MDS or acute leukemia evolving from MDS. Blood. 2012;120:1398-408.

20. Bödör C, Renneville A, Smith M, et al. Germ-line GATA2 p. THR354MET mutation in familial myelodysplastic syndrome with acquired monosomy 7 and ASXL1 mutation demonstrating rapid onset and poor survival. Haematologica. 2012;97:890-4.

21. Olopade O, Roulston D, Baker T, et al. Familial myeloid leukemia associated with loss of the long arm of chromosome 5. Leukemia. 1996;10:669-74.

22. Kawankar N, Rao Vundinti B. Cytogenetic abnormalities in myelodysplastic syndrome: an overview. Hematology. 2011;16:131-8.

23. Wilczynska A, Bushell M. The complexity of miRNA-mediated repression. Cell Death Differ. 2015;22:22-33.

[Downloaded from ibbj.org on 2026-09-15]

24. Pistritto G, Trisciuoglio D, Ceci C, et al. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging. 2016;8:603-19.
25. Erdogan B, Facey C, Qualtieri J, et al. Diagnostic microRNAs in myelodysplastic syndrome. Exp Hematol. 2011;39:915-26.
26. Di Leva G, Croce C M. miRNA profiling of cancer. Curr Opin Genet Dev. 2013;23:3-11.
27. Zhang Z, Huang L, Yu Z, et al. Let-7a functions as a tumor suppressor in Ewing's sarcoma cell lines partly by targeting cyclin-dependent kinase 6. DNA Cell Biol. 2014;33:136-47.
28. Merkerova M D, Krejcik Z, Votavova H, et al. Distinctive microRNA expression profiles in CD34&plus ;bone marrow cells from patients with myelodysplastic syndrome. Eur J Hum Genet. 2011;19:313-9.
29. Rhyasen G, Starczynowski D. Deregulation of microRNAs in myelodysplastic syndrome. Leukemia. 2012;26:13-22.
30. Danen-van Oorschot A A ,Kuipers J E, Arentsen-Peters S, et al. Differentially expressed miRNAs in cytogenetic and molecular subtypes of pediatric acute myeloid leukemia. Pediatr Blood Cancer. 2012;58:715-21.
31. Hussein K, Theophile K, Büsche G, et al. Aberrant microRNA expression pattern in myelodysplastic bone marrow cells. Leuk Res. 2010;34:1169-74.
32. Hussein K, Theophile K, Büsche G, et al. Significant inverse correlation of microRNA-150/MYB and microRNA-222/p27 in myelodysplastic syndrome. Leuke Res.2010;34:328-34
33. Zuo Z, Calin G A, de Paula H M, et al. Circulating microRNAs let-7a and miR-16 predict progression-free survival and overall survival in patients with myelodysplastic syndrome. Blood. 2011;118:413-5.
34. Takamizawa J, Konishi H, Yanagisawa K, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res. 2004;64:3753-6.
35. Johnson S M, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. Cell. 2005;120:635-47.
36. Volinia S, Calin G A, Liu C-G, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006;103:2257-61.
37. Cox A D, Der C J. The dark side of Ras: regulation of apoptosis. Oncogene. 2003;22:8999-9006.

[Downloaded from ibbj.org on 2026-09-15]