International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:7, No:1, 2013

MiRNAs as Regulators of Tumour Suppressor Expression

Olga A. Berillo, Gaukhar K. Baidildinova, and Anatoliy T. Ivashchenko

***Abstract*—**Tumour suppressors are key participants in the prevention of cancer. Regulation of their expression through miRNAs is important for comprehensive translation inhibition of tumour suppressors and elucidation of carcinogenesis mechanisms. We studies the possibility of 1521 miRNAs to bind with 873 mRNAs of human tumour suppressors using RNAHybrid 2.1 and E-RNAhybrid programmes. Only 978 miRNAs were found to be translational regulators of 812 mRNAs, and 61 mRNAs did not have any miRNA binding sites. Additionally, 45.9% of all miRNA binding sites were located in coding sequences (CDSs), 33.8% were located in 3′ untranslated region (UTR), and 20.3% were located in the 5′UTR. MiRNAs binding with more than 50 target mRNAs and mRNAs binding with several miRNAs were selected. Hsa-miR-5096 had 15 perfectly complementary binding sites with mRNAs of 14 tumour suppressors. These newly indentified miRNA binding sites can be used in the development of medicines (anti-sense therapies) for cancer treatment.

***Keywords*—**Exonic miRNA, intergenic miRNA, intronic miRNA, tumor suppressor.

I. Introduction

TUMOUR suppressors control cell proliferation and prevent the exit of cells from differentiation processes [1]. Some of these genes participate in apoptosis and are very important in cell cycle control. Loss of suppressor activity causes uncontrollable cell proliferation [2]. The function of tumour suppressors can decrease through interactions with miRNAs that influence the expression of these genes. Indeed, differential expression of miRNAs has been demonstrated in cancer patients compared to healthy individuals [3].

MiRNAs are short noncoding RNAs with a length of about 22 nucleotides that promote the suppression of translation [4]. MiRNAs can be intragenic or intergenic (ig-miRNA). Intragenic miRNAs are localised within intronic (in-miRNA) or exonic (ex-miRNA) regions of their host genes [5], and some are found on the boundaries between exons and introns [6]. Several miRNAs regulate different cell processes, including DNA repair, apoptosis, the oxidative stress response [7], cell proliferation, migration, invasion, and anti-apoptotic pathways [8]. Changes in miRNA concentrations correlate with clinical stages of cancer [9].

Many researchers have suggested that miRNAs bind only with 3′UTRs of mRNAs [10]-[13]; however, some studies have described miRNA binding sites in the 5′UTRs and CDSs of mRNAs [14]-[16]. Data on the locations of miRNA binding sites could improve our understanding of the interactions between miRNAs and mRNAs. The effects of many miRNAs on mRNA translation remain unknown [17].

It is known that many miRNAs can be found in all biological liquids of organisms [18], [19]. For example, the serum concentrations of some miRNA can change considerably during the development of cancer [20]. Thus, it is important to identify the specific miRNAs that bind to mRNAs of tumour suppressors. Changes in the concentrations of these miRNAs can act as a signal of the malignant degenerations of cells. MiRNAs can be used as markers for early diagnostics and treatment of tumours [21]. Currently, about 1500 miRNAs are known, but many miRNAs and their target mRNAs have not been well studied. For this study, we selected tumour suppressor genes to search for additional miRNA binding sites. The aims of our work are follows: (a) to reveal features of miRNA interactions with different regions of tumour suppressor mRNAs; (b) to select miRNAs binding with many mRNAs; (c) to identify mRNAs binding with many miRNAs; and (d) to reveal miRNA binding sites with perfect complementarity.

II. Materials and Methods

A list of 873 tumour suppressor genes was borrowed from the Memorial Sloan-Kettering Cancer Center (http://cbio.mskcc.org/CancerGenes/Select.action), and their nucleotide sequences were taken from GenBank (http://www.ncbi.nlm.nih.gov). Nucleotide sequences of miRNAs were obtained from the miRBase (http://www.mirbase.org).

The miRNA-Finder 2.2 programme (http://sites.google.com/site/malaheenee/software/) was used for identification of miRNA origins: intronic, exonic, or intergenic. Schemes of miRNA:mRNA complexes were found, and their free energies of miRNA interactions (ΔGs) was calculated using the RNAHybrid 2.1 programme. The $\Delta G/\Delta G_m$ ratio, significance ($p < 0.0005$), and mRNA regions (5′UTR, CDS, or 3′UTR) where miRNA binding sites are located were found using the E-RNAhybrid 2.1 programme (http://sites.google.com/site/malaheenee/software/). The $\Delta G/\Delta G_m$ (%) value was calculated, where ΔG_m was the free

O. A. Berillo is with the National Nanotechnology Laboratory of Open Type al-Farabi KazNU, 050038, Almaty, Kazakhstan (corresponding author, Tel.: +7 (727) 377 33 29, fax: 8 (727)3773202, e-mail: devolia18@mail.ru).

G. K. Baidildinova is with the National Nanotechnology Laboratory of Open Type al-Farabi KazNU, 050038, Almaty, Kazakhstan (e-mail: baidildag@mail.ru).

A. T. Ivashchenko is with the National Nanotechnology Laboratory of Open Type al-Farabi KazNU, 050038, Almaty, Kazakhstan (e-mail: a_ivashchenko@mail.ru).

energy of the miRNA binding site having perfect complementary with mRNA. The densities of miRNA binding sites located in the 5′UTR, CDS, and 3′UTR were calculated as number of sites divided by the length of the site and multiplied by 10^3 (s/l). The level of significance was calculated on the basis of free energy (ΔG) and its standard deviation. Significant miRNA binding sites were selected ($p < 0.0005$).

III. Results and Discussion

The binding sites of 785 ig-miRNAs, 686 in-miRNAs, and 49 ex-miRNAs were investigated within the 5′UTRs, CDSs, and 3′UTRs of 873 for human tumour suppressor mRNAs. Only 978 of 1521 investigated miRNAs were found to be translation regulators of 812 mRNAs. The majority of studied miRNA binding sites were located in the CDSs of mRNAs (45.9%). About one-third of all binding sites (33.8%) were located in 3′UTRs, and 20.3% were located in 5′UTRs. A total of 3571 binding sites were formed with ig-miRNAs, 2662 were formed with in-miRNAs, and 272 were formed with ex-miRNAs. Nineteen miRNAs were encoded in introns of genes and intergenic regions. They participated in forming of 93 binding sites that were found in 87 mRNAs. These genes may undergo difficult regulation by miRNAs, because ig-miRNAs can be expressed independently, the majority of in-miRNAs depend on splicing of their host genes. MiR-1285 was encoded in the intergenic region of the second chromosome and in intron of the *KRIT1* gene. Binding sites for miR-1285 were located in UTRs of mRNAs (27 in the 3′UTR and 3 in the 5′UTR).

A total of 511 ig-miRNAs participated in the regulation of 811 genes; 17 ig-miRNAs had 51–110 target genes (Table I). For example, miR-4472, with an average GC-content (55.5%), had 110 binding sites, 61 of which were localised in the CDS, 27 of which were located in the 3′UTR, and 22 of which were located in the 5′UTR. While miR-4466, with high GC-content (94.4%), had many binding sites (29) in the 5′UTR, 15 binding sites were located in the CDS and 7 were located in the 3′UTR. MiR-1273, with low GC-content (48.8%), had 57 mRNA binding sites. The majority of these sites (34) were located in the CDS, 17 miRNA sites were found in the 3′UTR, and the remaining binding sites were found in the 5′UTR. These examples show that miRNAs with high GC-content have binding sites in 5′UTRs, while miRNAs with low GC-content form binding sites in 3′UTRs.

A total of 444 in-miRNAs participated in forming binding sites with 710 mRNAs. Some in-miRNAs contained between 52 and 80 target genes (Table I). For example, in-miR-1268b, which has high GC-content, 80 sites located in the 5′UTR (26), CDS (32), and 3′UTR (22) were found.

Forty-three ex-miRNAs participated in the regulation of 202 genes. MiR-1306, with average GC-content (61.1%), had 31 binding sites, 5 of which were situated in the 5′UTR, 22 of which were located in the CDS, and 2 of which were found in the 3′UTR. The CDS of *WNK2* mRNA had 3 miR-1306 sites. Expression of the *BAI1* gene was regulated by ex-miR-4687-5p, ex-miR-4707-5p, and ex-miR-4800-5p, which bound to the CDS, and ex-miR-4800-3p which bound with the 3′UTR. Many mRNAs of these 202 genes had only a single miRNA binding site.

Several genes had multiple miRNA binding sites in their mRNAs. For example, mRNA for *PKD1* (polycystic kidney disease 1), with a length of 14138 nucleotides, had 56 miRNA binding sites, including 30 ig-miRNAs, 22 in-miRNAs, and 4 ex-miRNAs. PKD1 participates in anatomical structure morphogenesis, calcium-independent cell-matrix adhesion, neuropeptide signalling pathways, etc. The binding sites for miR-125b-1*, miR-3178, and miR-4787-5p were located in the 5′UTR, including 50 sites in the CDS, and miR-4492, miR-466, and miR-608 binding sites were located in the 3′UTR of *PKD1*. The densities of miRNA sites in the 5′UTR, CDS, and 3′UTR were 14.3 s/l, 3.8 s/l, and 2.5 s/l, respectively.

TABLE I
Quantity of miRNA Binding Sites with Different Regions of mRNAs

miRNA	5'UTR	CDS	3'UTR	GC	miRNA	5'UTR	CDS	3'UTR	GC
Ig-miR-302f	3	36	23	29.4	Ig-miR-4455	23	46	33	41.2
In-miR-1268	20	32	24	83.3	Ig-miR-4456	16	42	29	52.9
In-miR-1268b	26	32	22	80.0	Ig-miR-4466	29	15	7	94.4
In-miR-1273f	7	3	53	48.0	Ig-miR-4472	22	61	27	55.6
Ig-miR-1279	6	34	17	29.4	Ig-miR-4481	15	33	15	64.7
Ig-miR-1587	19	39	7	70.0	Ig-miR-4483	9	41	17	58.8
Ig-miR-3195	24	31	2	88.2	Ig-miR-4507	16	29	8	75.0
Ig-miR-4279	26	18	18	68.8	Ig-miR-4508	43	27	2	94.1
In-miR-4297	23	27	13	56.3	Ig-miR-4710	8	29	20	66.7
Ig-miR-4307	16	25	24	31.6	In-miR-5095	1	2	49	61.9
Ig-miR-4328	5	33	16	47.1	In-miR-5096	1	6	60	52.4
Ig-miR-4443	17	38	21	52.9					

GC-content of miRNAs were calculated in percent.

The characteristics of miRNA binding with 5′UTRs, CDSs, and 3′UTRs were studied. A total of 210 ig-miRNAs, 173 in-miRNAs, and 19 ex-miRNAs contained binding sites targeting 5′UTRs despite their small lengths. Some miRNAs had multiple binding sites within certain regions of mRNA. Forty-three of 72 sites for miR-4508 were located in 5′UTRs of 38 mRNAs. *BCR*, *DRAM1*, and *EPHB4* mRNAs contained 2 binding sites each with miR-4508 and *SMAD3* mRNA contained 3 miR-4508 binding sites in its 5′UTR.

A total of 2959 miRNA binding sites were located in 676 CDSs of mRNAs. Additionally, 138 CDSs of mRNAs could bind to 37 ex-miRNAs (116 sites). CDSs were more highly conserved than 5′UTRs and 3′UTRs, and their miRNAs binding sites were more highly conserved as well [22]. The CDSs of 561 target genes had 1716 binding sites with 367 ig-miRNAs. The numbers of intronic and intergenic miRNAs were approximately equal, but the number of ig-miRNA binding sites was 1.6 times greater than the number of in-miRNA binding sites. In-miRNAs (335) participated in the formation of 1068 sites located in 478 CDSs. Ig-miR-4472 regulated the translation of 55 target genes through 61 miRNA binding sites. CDSs of *JUP*, *MN1*, *NOTCH1*, *SCRIB*, and *WNK2* genes had 2 binding sites with ig-miR-4472. The CDS of *LAMA5* had 38 binding sites: 24 for ig-miRNAs, 14 for in-miRNAs, and 1 site with mixed origin. Ig-miR-4483 had 3 binding sites within this region.

A total of 2150 binding sites were found in 3′UTRs, and 989 of these sites were formed by 242 in-miRNAs to regulate the translation of 367 mRNAs. Approximately equal numbers of intergenic and intronic miRNAs participated in the regulation of tumour gene expression via binding with the 3′UTR. Additionally, 428 mRNAs had 1024 binding sites which were formed by 300 ig-miRNAs. Only 29 ex-miRNAs could bind with 66 mRNAs, forming 73 binding sites. Fifty-seven sites were formed between 43 mRNAs and 10 miRNAs with mixed origin (intronic and intergenic). The 3′UTR of *SPN* had 35 binding sites with 27 miRNAs; among them, in-miR-5095 had 3 binding sites, and in-miR-1226, in-miR-1273, ig/in-miR-1285, ig-miR-297, in-miR-5096, and in-miR-574-5p each had 2 binding sites. Some miRNAs had more binding sites in the 3′UTR. For example, in-miR-5096 had 60 binding sites with 47 mRNAs. Some genes had several miRNA binding sites in their 3′UTRs. For example, *IKZF3*, *MDM4*, and *VPS53* each had 3 miR-5096 binding sites and *C12orf5*, *IRF1*, *KIF1B*, *RBBP5*, *SMYD4*, *SPN*, and *VHL* each had 3 miR-5096 binding sites. In-miR-1273f had binding sites with *MDM4* which began with nucleotides 2201, 3069, 4901, 7177, and 9423.

Twenty miRNA binding sites with mRNAs had perfect nucleotide complementarity (Table II); from this connection, it was possible to name these miRNAs “natural siRNAs”. It is necessary to confirm these miRNA binding sites by experimental investigation, and it will be interesting to identify whether they will promote splitting of target mRNAs similar to true siRNAs? Fifteen of these sites belong to miR-5096, with an average GC-content of 52.4%. Precursor miR-5096 was encoded in the first intron of bone morphogenetic protein 2 inducible kinase (*BMP2K*), which plays an important role in the development of the skeleton. The majority of intronic and exonic miRNAs are expressed together with their host genes, encoded as precursors miRNAs. These miRNAs provide connections between host genes and target genes through intragenic miRNAs. Changes in host gene expression lead to changes in intronic and exonic miRNA expression, which influence the translation of their target genes. The expression of *BMP2K* mRNA influences the translation of 67 tumour suppressor mRNAs via in-miR-5096. *IKZF3*, *MDM4*, and *VPS53* mRNAs had 3 binding sites with miR-5096, and *C12orf5*, *IRF1*, *KIF1B*, *RBBP5*, *SMYD4*, *SPN*, and *VHL* mRNAs has 2 miR-5096 binding sites. Sialophorin (SPN) is a receptor found on the outer cell membrane that participates in signal transduction induced by the extracellular apoptotic signalling pathway. *SPN* mRNA had 2 perfectly complementary binding sites with in-miR-5096 in the 3′UTR (Table II). The existence of more than one binding site will promote conservation of translational regulation, even if some of the sites have mutations. Several binding sites in certain mRNAs can enhance the suppressive effects of miRNAs.

Schemes of interactions between miR-5096 and the 3′UTR of tumour suppressors are presented in Table III. Their free energy (ΔG) changed slightly from -45.2 to -43.4 kcal/mol in connection with several nucleotide replacements of G-C pairs

TABLE II
CHARACTERISTICS OF PERFECT COMPLEMENTARY MIRNA BINDING SITES WITH MRNAS

Target gene	miRNA	mRNA region	p<	Gene origin
ARL11	in-miR-5096	3'UTR	4.0e-05	*BMP2K*
BRCA1	in-miR-5096	3'UTR	4.0e-05	*BMP2K*
C12orf5	in-miR-5096	3'UTR	4.0e-05	*BMP2K*
CKAP4	ig-miR-507	CDS	3.8e-05	-
EPHB2	ig-miR-4253	CDS	5.0e-05	-
IL17RD	in-miR-5096	3'UTR	3.6e-05	*BMP2K*
IRF1	in-miR-5096	3'UTR	4.0e-05	*BMP2K*
KIF1B	in-miR-5096	3'UTR	4.0e-05	*BMP2K*
KRAS	in-miR-1273f	3'UTR	4.6e-05	*SCP2*
LIMD1	in-miR-5096	3'UTR	3.1e-05	*BMP2K*
PPP2R1B	in-miR-5096	3'UTR	3.6e-05	*BMP2K*
RASSF6	in-miR-5096	3'UTR	4.0e-05	*BMP2K*
RBBP4	in-miR-5095	3'UTR	3.8e-05	*SCP2*
SLC4A1	in-miR-5096	3'UTR	4.0e-05	*BMP2K*
SMYD4	in-miR-5096	3'UTR	4.0e-05	*BMP2K*
SPN	in-miR-5096	3'UTR	3.6e-05	*BMP2K*
SPN	in-miR-5096	3'UTR	4.0e-05	*BMP2K*
VPS53	in-miR-5096	3'UTR	4.2e-05	*BMP2K*
XDH	ig-miR-4328	CDS	6.4e-05	-
ZC3H12D	in-miR-5096	3'UTR	4.0e-05	*BMP2K*

TABLE III
INTERACTION SCHEMES OF MIR-5096

BRCA1, RASSF6, C12orf5, SMYD, ARL11, SPN, IRF1, SLC4A1, KIF1B, ZC3H12D mRNAs.

```
mRNA      5' A                    C 3'
               GCCUGGCCAACAUGGUGAAAC
               CGGACUGGUUGUACCACUUUG
miRNA     3'                       5'
```

IL17RD, SPN, PPP2R1B mRNAs.

```
mRNA      5' A                    C 3'
               GCCUGACCAACAUGGUGAAAC
               CGGACUGGUUGUACCACUUUG
miRNA     3'                       5'
```

```
LIMD1 mRNA   5' A                     A 3'
                 GCCUGACCAACAUGGUGAAAC
                 CGGACUGGUUGUACCACUUUG
miRNA        3'                        5'
```

```
VPS53 mRNA   5' A                     C 3'
                 GCCUGGCCGACAUGGUGAAAC
                 CGGACUGGUUGUACCACUUUG
miRNA        3'                        5'
```

The bold print allocated with changed nucleotides in comparison to first scheme

by G-U in different mRNAs. These schemes of miRNA:mRNA interactions belong to the complementary type, which was recently found in mRNAs of oncogenes [15]. Other miRNA binding sites with $p < 0.0005$ do not have perfect complementarity and belong to the 5′-dominant canonical and 3′-compensatory types. Increased concentrations of miRNAs, repressing the expression of gene suppressors, may indicate the promotion of carcinogenesis.

IV. CONCLUSION

MiRNAs have binding sites with several distinct mRNAs of tumour suppressor genes. Significant miRNA binding sites ($p < 0.0005$) are located in 5′UTRs, CDSs, and 3′UTRs of mRNAs. MiRNAs that repress the translation of studied genes can be considered oncogenes. Changing the concentration of relevant miRNAs may have potential use in anti-sense therapy in the treatment of cancer. Twenty miRNA binding sites with perfect complementarity were found in the current study. It is possible that these binding sites promote complete splitting of target mRNAs. We recommend that these newly described miRNA binding sites should be confirmed experimentally. These miRNAs can be used for diagnostics and the treatment of cancers.

ACKNOWLEDGMENT

We thank V. Khailenko for the program miRNAFinder 2.2 and E-RNAhybrid 2.1.This study was supported by grant of Ministry of Education and Science, Kazakhstan Republic.

REFERENCES

[1] A. Chow, C.L. Arteaga, S.E. Wang, “When tumor suppressor TGFβ meets the *HER2* (*ERBB2*) oncogene”, *J Mammary Gland Biol Neoplasia,* vol. 2, no. 16, pp. 81-8. 2011.

[2] A. Andersen, D.A. Jones, “APC and DNA Demethylation in Cell Fate Specification and Intestinal Cancer”, *Adv Exp Med Biol*, no. 754, pp. 167-77. 2013.

[3] M.A. Cortez, C. Bueso-Ramos, J. Ferdin, G. Lopez-Berestein, A.K. Sood, G.A. Calin, “MicroRNAs in body fluids-the mix of hormones and biomarkers”, *Nat Rev Clin Oncol*, vol. 8, no. 8, pp. 467-77. 2011.

[4] M. Ul Hussain, “Micro-RNAs (miRNAs): genomic organisation, biogenesis and mode of action”, *Cell Tissue Res,* vol. 2, no. 349, pp. 405-13. 2012.

[5] Y.K. Kim, V.N. Kim, “Processing of intronic microRNAs”, *Embo J*, no. 26, pp. 775-783. 2007.

[6] A. Rodriguez, S. Griffiths-Jones, J.L. Ashurst, A. Bradley, “Identification of mammalian microRNA host genes and transcription units”, *Genome Res,* no. 14, pp. 1902–1910. 2004.

[7] A. Allegra, A. Alonci, S. Campo, G. Penna, A. Petrungaro, D. Gerace, C. Musolino, “Circulating microRNAs: New biomarkers in diagnosis, prognosis and treatment of cancer”, *Int J Onco*l, 1647. 2012. doi: 10.3892/ijo.2012.1647.

[8] Y. Zhang, S. Takahashi, A. Tasaka, T. Yoshima, H. Ochi, K. Chayama, “Involvement of microRNA-224 in cell proliferation, migration, invasion and anti-apoptosis in hepatocellular carcinoma”, *J Gastroenterol Hepatol*, pp. 1440-1746. 2012. doi: 10.1111/j.1440-1746.2012.07271.x.

[9] H. Cheng1, L. Zhang, D.E. Cogdell, H. Zheng, A.J. Schetter, M. Nykter, C. Curtis. Harris, K. Chen, S.R. Hamilton, W. Zhang, “Circulating Plasma MiR-141 Is a Novel Biomarker for Metastatic Colon”, *Cancer and Predicts Poor Prognosis*, vol. 6, no. 3, pp. E17745. 2011.

[10] 10 D. Long, C.Y. Chan, Y. Ding, “Analysis of microRNA-target interactions by a target structure based hybridization model”, *Pac Symp Biocomput,* pp. 64-74. 2008.

[11] D. Goldoni, J.M. Yarham, M.K. McGahon, A. O'Connor, J. Guduric-Fuchs, K. Edgar, D.M. McDonald, D.A. Simpson, A. Collins, “A novel dual-fluorescence strategy for functionally validating microRNA targets in 3-prime untranslated regions: regulation of the inward rectifier potassium channel Kir2.1 by miR-212”, *Biochem J*. 2012.

[12] B.L. Brewster, F. Rossiello, J.D. French, S.L. Edwards, M. Wong, A. Wronski, P. Whiley, N. Waddell, X. Chen, B. Bove, Kconfab, J.L. Hopper, E.M. John, I. Andrulis, “Identification of fifteen novel germline variants in the BRCA1 3'UTR reveals a variant in a breast cancer case that introduces a functional miR-103 target site”, *Hum Mutat*. 2012. doi: 10.1002/humu.22159.

[13] J. Satoh, H. Tabunoki, “Comprehensive analysis of human microRNA target networks”, *BioData Mining,* no. 4, pp. 17. 2011.

[14] X. Zhou, X. Duan, J. Qian, F. Li, “Abundant conserved microRNA target sites in the 5'-untranslated region and coding sequence”, *Genetica,* vol. 2, no. 137, pp. 159-64. 2009.

[15] A. Issabekova, O. Berillo, M. Regnier, A. Ivashchenko, “Interactions of intergenic microRNAs with mRNAs of genes involved in carcinogenesis”, *Biomedical Informatics,* vol. 11, no. 8, pp. 513-518. 2012.

[16] F. Moretti, R. Thermann, M. Hentze, “Mechanism of translational regulation by miR-2 from sites in the 5' untranslated region or the open reading frame”, *RNA*, no. 16, pp. 2493–2502. 2010.

[17] I. Lee, S.S. Ajay, J.I. Yook, H.S. Kim, S.H. Hong, N.H. Kim, S.M. Dhanasekaran, A.M. Chinnaiyan, B.D. Athey, “New class of microRNA targets containing simultaneous 5′-UTR and 3 ′-UTR interaction sites”, *Genome Res,* vol. 7, no. 19, pp. 1175-1183. 2009.

[18] G. Tzimagiorgis, E.Z. Michailidou, A. Kritis, A.K. Markopoulos, S. Kouidou, “Recovering circulating extracellular or cell-free RNA from bodily fluids”, *Cancer Epidemiol*, vol. 6, no. 35, pp. 580-9. 2011.

[19] J.A. Weber, D.H. Baxter, S. Zhang, D.Y. Huang, K.H. Huang, M.J. Lee, D.J. Galas, K. Wang, “The microRNA spectrum in 12 body fluids”, *Clin Chem*, vol. 11, no. 56, pp. 1733-41. 2010.

[20] R. Albulescu, M. Neagu, L. Albulescu, C. Tanase, "Tissular and soluble miRNAs for diagnostic and therapy improvement in digestive tract cancers", *Expert Rev Mol Diagn,* vol. 1, no. 11, pp. 101-20. 2011.
[21] M.V. Iorio, C.M. Croce, "MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review", *EMBO Mol Med*, vol. 3, no. 4, pp. 143-59. 2012.
[22] A. T. Ivashchenko, A. S. Issabekova, O. A. Berillo "Peculiarities of miR-1279 binding sites in CDS of PTPN12, MSH6 and ZEB1 oncogenes of human and animal". unpublished.