MicroRNA Signature in Myelodysplastic Syndrome

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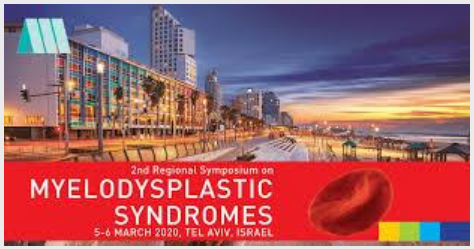

Disclosures

No affiliations

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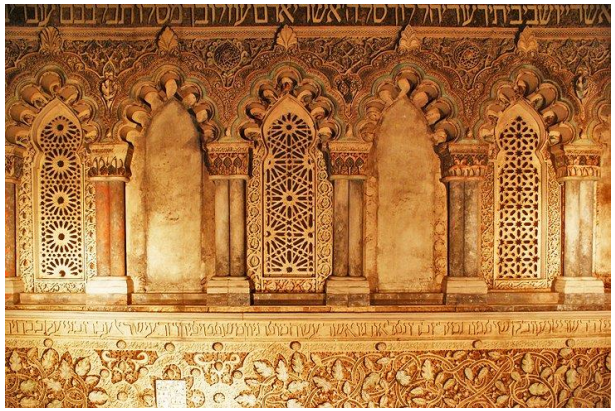


Is bone marrow examination always necessary to establish the diagnosis o myelodysplastic syndromes? A propos non-invasive diagnostic model
Article *in* [Leukemia and Lymphoma](#) 59(9):1-6 · January 2018

ASH 50th anniversary review

Myelodysplastic syndromes

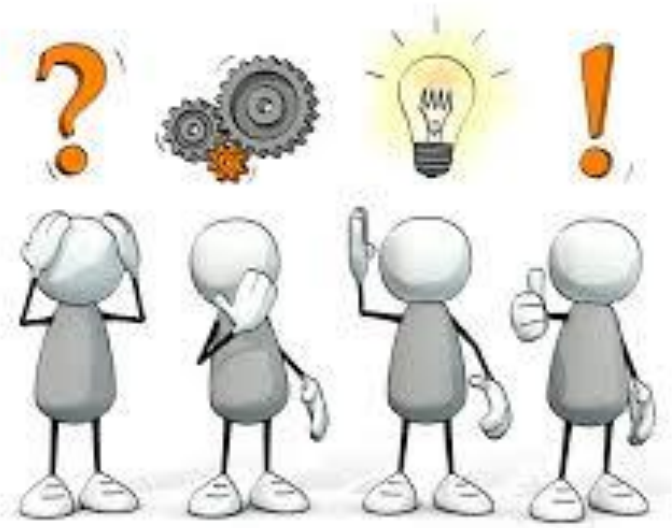
Stephen D. Nimer[1]

A burning question that faces the field is: what is missing from our picture of MDS? Whereas this question is relevant for nearly all malignancies that we treat, as we make great strides in understanding hematopoietic stem cell biology, we should gain further insights into MDS. The FDA has approved 3 effective therapies in the past 3 to 4 years; thus, the future looks bright. Hopefully, the study of MDS will attract many talented clinical and scientific investigators in the future, and we can unravel more of the complexities of this group of disorders.

After long way…

- Increased incidence of MDS due to improved diagnostic methods and better disease awareness
- The improvement in survival due to better availability of therapeutic agents
- Ameliorated management and new treatment options
- More personalized approaches

But, are we really satisfied with the results?



LETTER TO THE EDITOR

Survival trends in primary myelodysplastic syndromes: a comparative analysis of 1000 patients by year of diagnosis and treatment

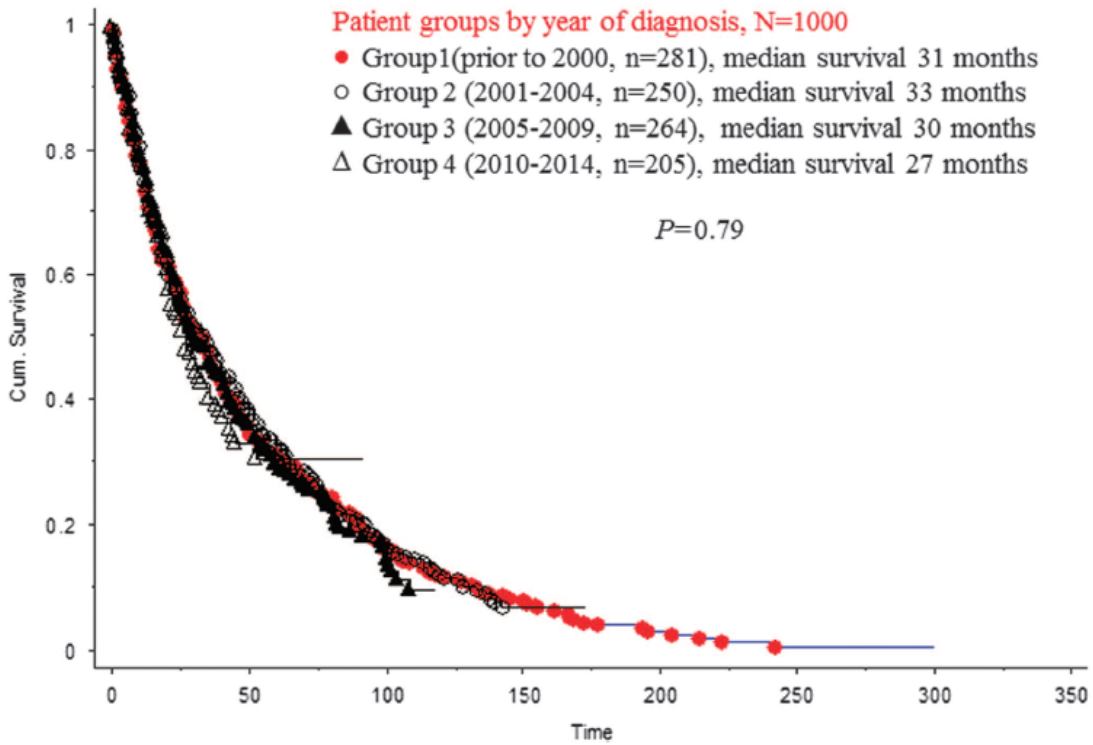


Figure 1. Survival trends among 1000 patients with primary MDS stratified by year of diagnosis.

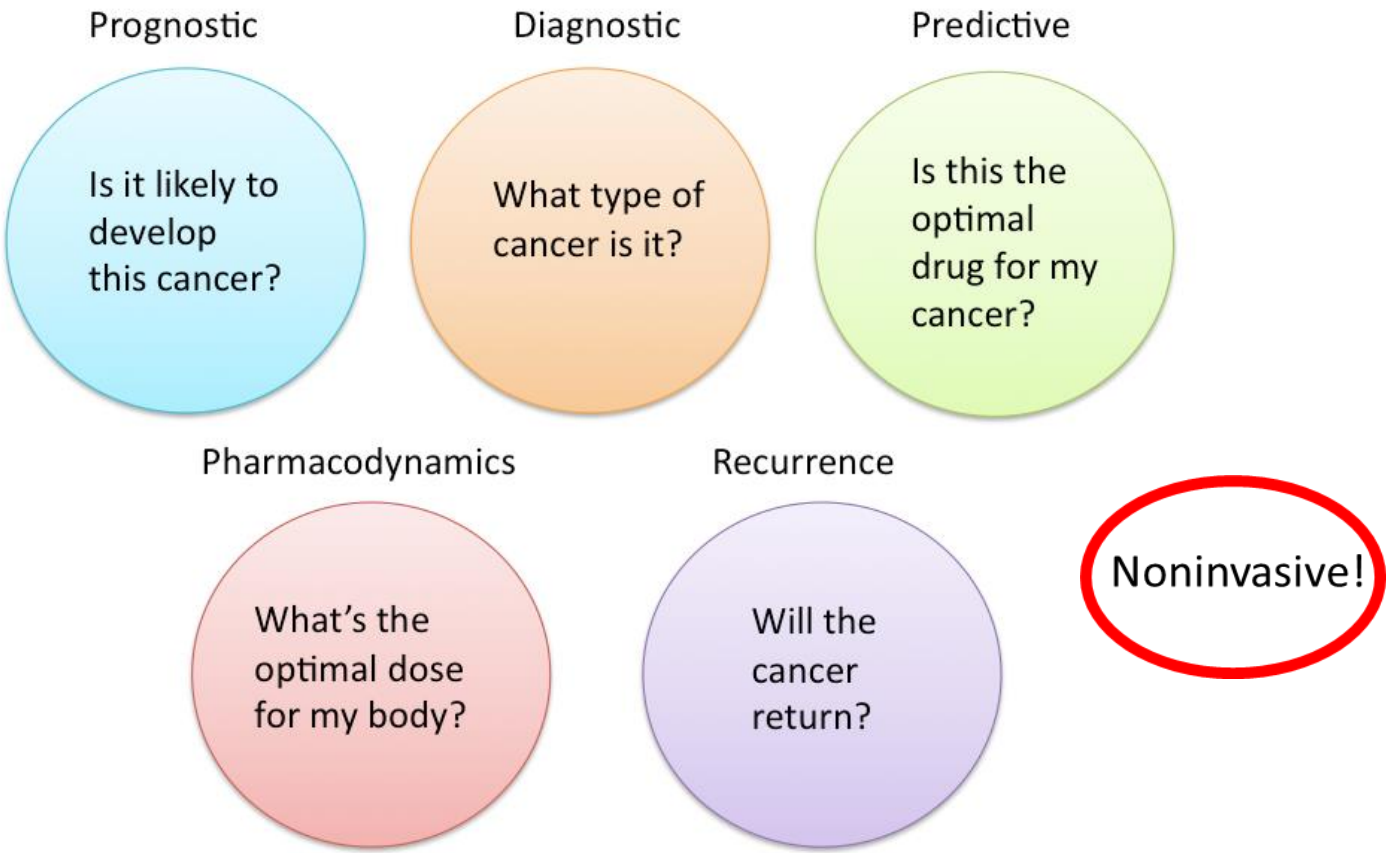
Blood Cancer Journal (2016) **6**, e414; doi:10.1038/bcj.2016.23

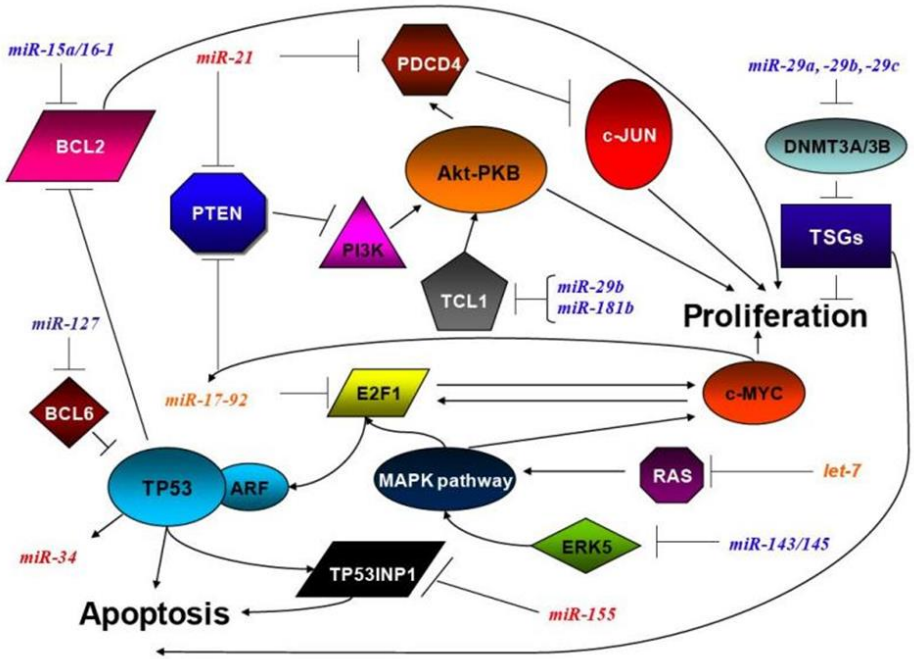
How to prolong OS?

- Earlier diagnosis?
- Better follow up?
- More efficacious therapies?
- Earlier detection of progression?

Need for new targets and molecules involved in cancer pathogenesis?

Questions for cancer biomarker?

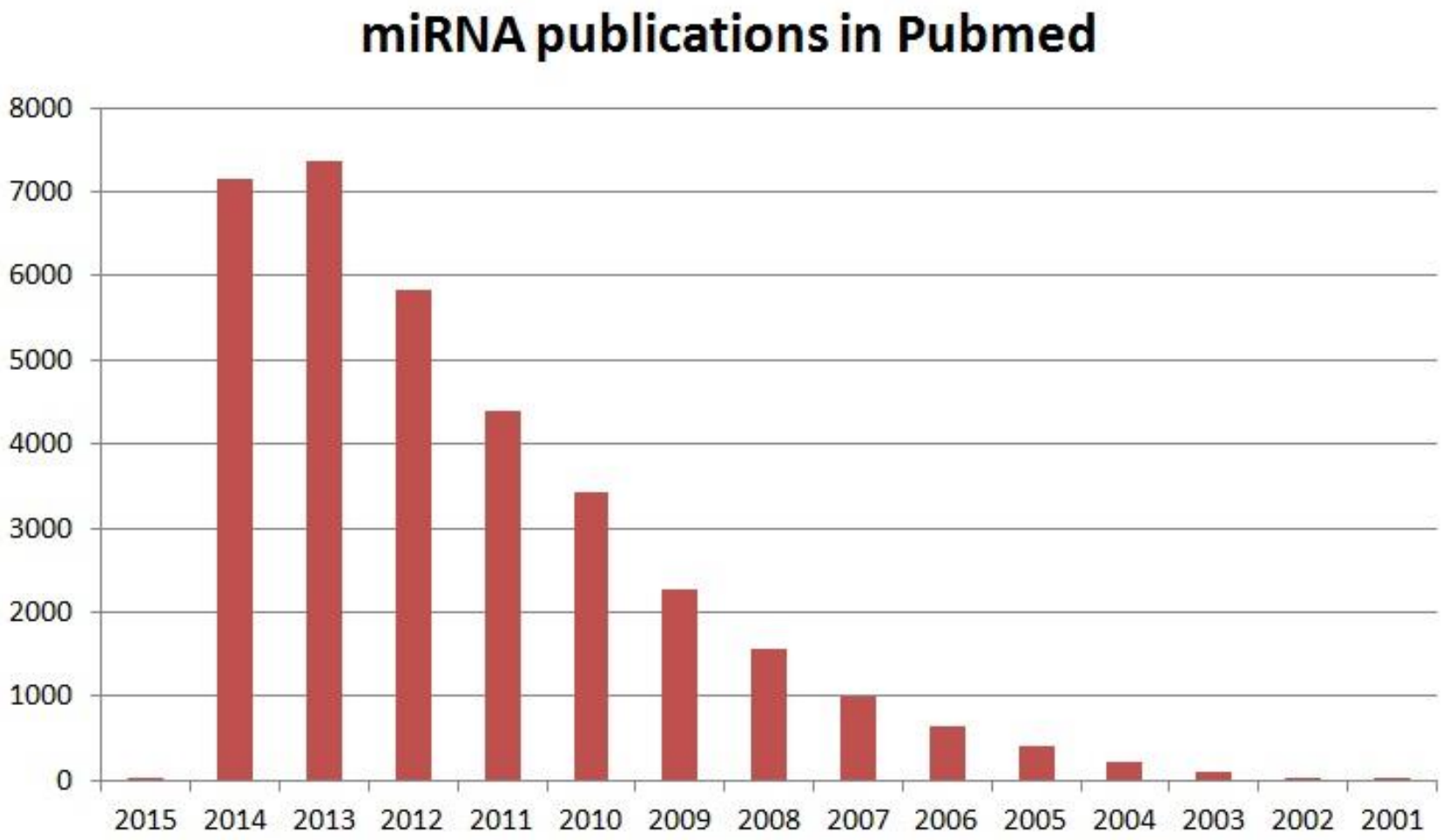




Nature 2011;478:64-9

- non-coding RNAs of 19-22 nucleotides in length that regulate gene expression at the posttranscriptional level by binding to mRNA of their cognate target genes and participate in numerous biological processes such as cell proliferation, differentiation, development, metabolism, survival and hematopoiesis

MicroRNA (miRNA)



https://www.biosyn.com/tew/micrornas-or-mirnas-as-cancer-biomarkers.aspx

PubMed ("MicroRNAs"[Mesh]) AND "Myelodysplastic Syndromes"[Mesh]

Format: Summary **Sort by**: Most Recent **Per page**: 20

Search results

Items: 1 to 20 of 46

Filters activated: published in the last 5 years, Clear all to show 110 items.

1. [Expression and Function of miR-99a-5p in Bone Marrow of Patients with MDS].
LE Y, Zhang XP, Xiong YQ, Li HY, Zhao WH, Long Y, Luo J, Cheng P, Liu ZF.
Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2019 Feb;27(1):134-140. doi: 10.7534/j.issn.1009-2137.2019.01.022. Chinese.
PMID: 30738460

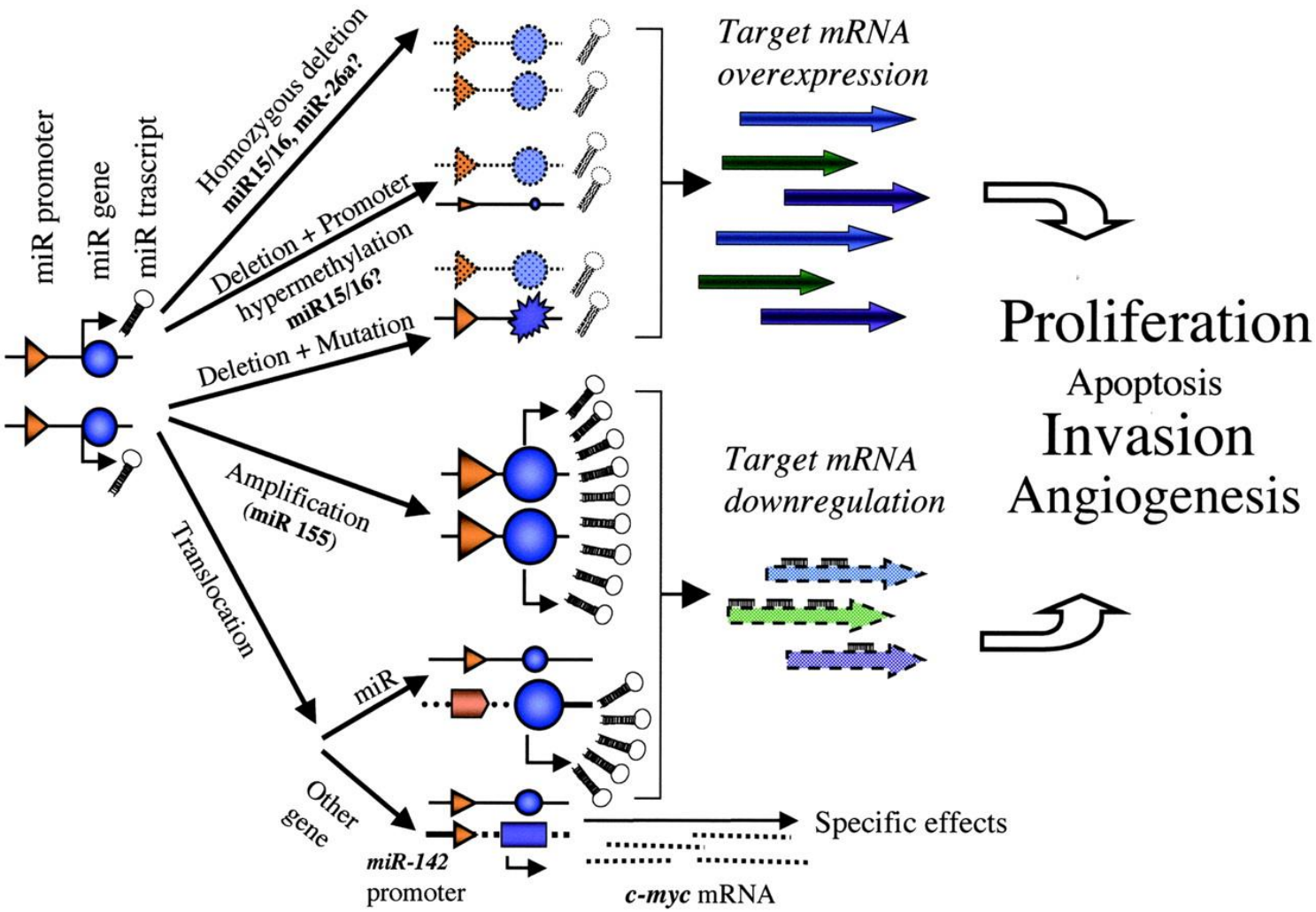
2. Molecular pathogenesis of **myelodysplastic syndromes** with deletion 5q.
Lee JH, List A, Sallman DA.
Eur J Haematol. 2019 Mar;102(3):203-209. doi: 10.1111/ejh.13207. Epub 2019 Jan 16. Review.
PMID: 30578738

3. Circulating microvesicles are less procoagulant and carry different miRNA cargo in myelodysplasia.
Enjeti AK, Ariyarajah A, D'Crus A, Riveros C, Seldon M, Lincz LF.
Blood Cells Mol Dis. 2019 Feb;74:37-43. doi: 10.1016/j.bcmd.2018.11.001. Epub 2018 Nov 7.
PMID: 30454964 **Free Article**

4. *SKI* controls MDS-associated chronic TGF-β signaling, aberrant splicing, and stem cell fitness.
Muench DE, Ferchen K, Velu CS, Pradhan K, Chetal K, Chen X, Weirauch MT, Colmenares C, Verma A, Salomonis N, Grimes HL.
Blood. 2018 Nov 22;132(21):e24-e34. doi: 10.1182/blood-2018-06-860890. Epub 2018 Sep 24.
PMID: 30249787 **Free PMC Article**

MicroRNA-143 increases cell apoptosis in myelodysplastic syndrome through the Fas/FasL pathway both in vitro and in vivo.

MiRs as cancer players.



George Adrian Calin et al. PNAS 2004;101:9:2999-3004

PNAS

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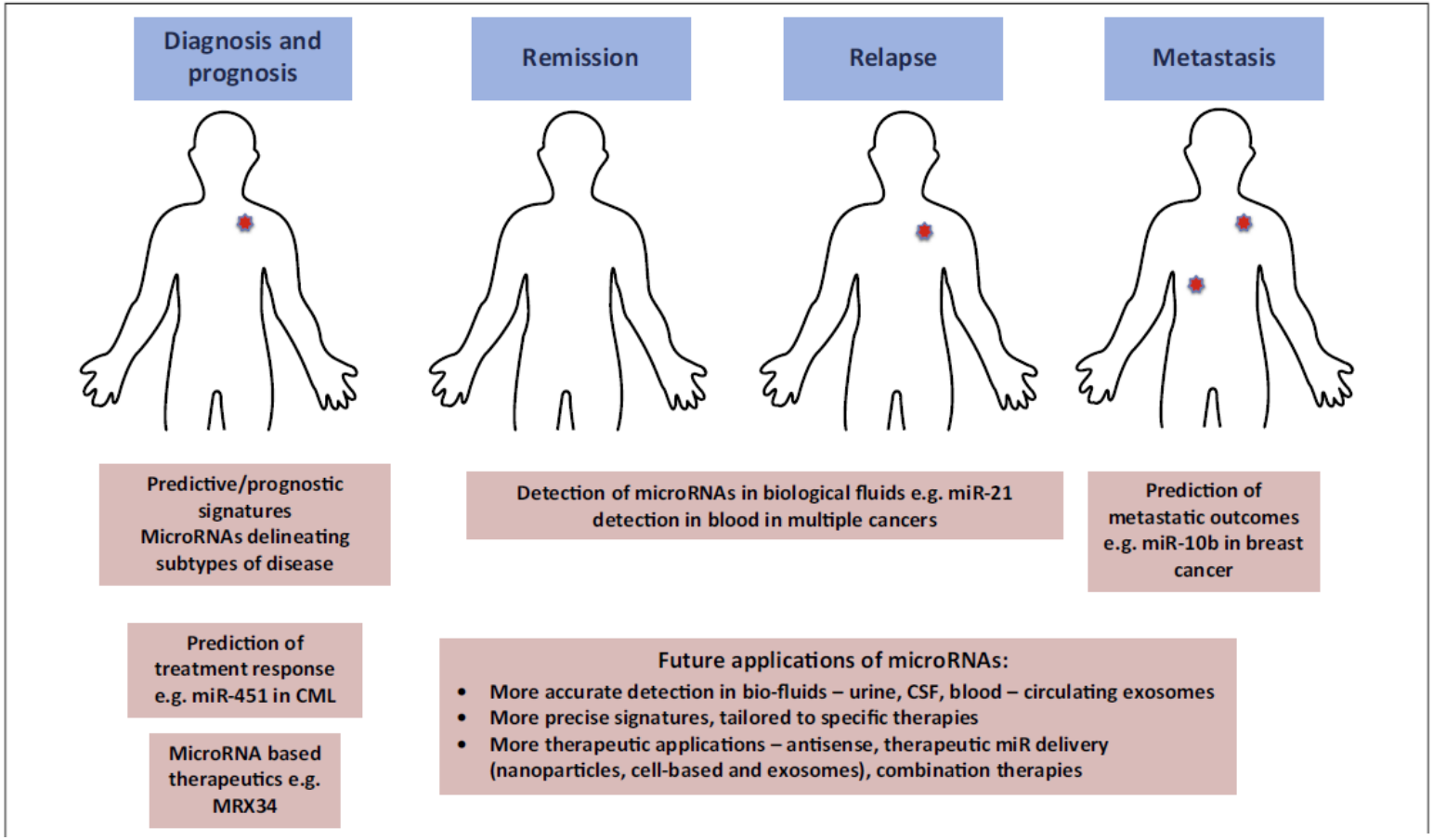
MicroRNAs in cancer: biomarkers, functions and therapy



Trends in Molecular Medicine, August 2014, Vol. 20, No. 8

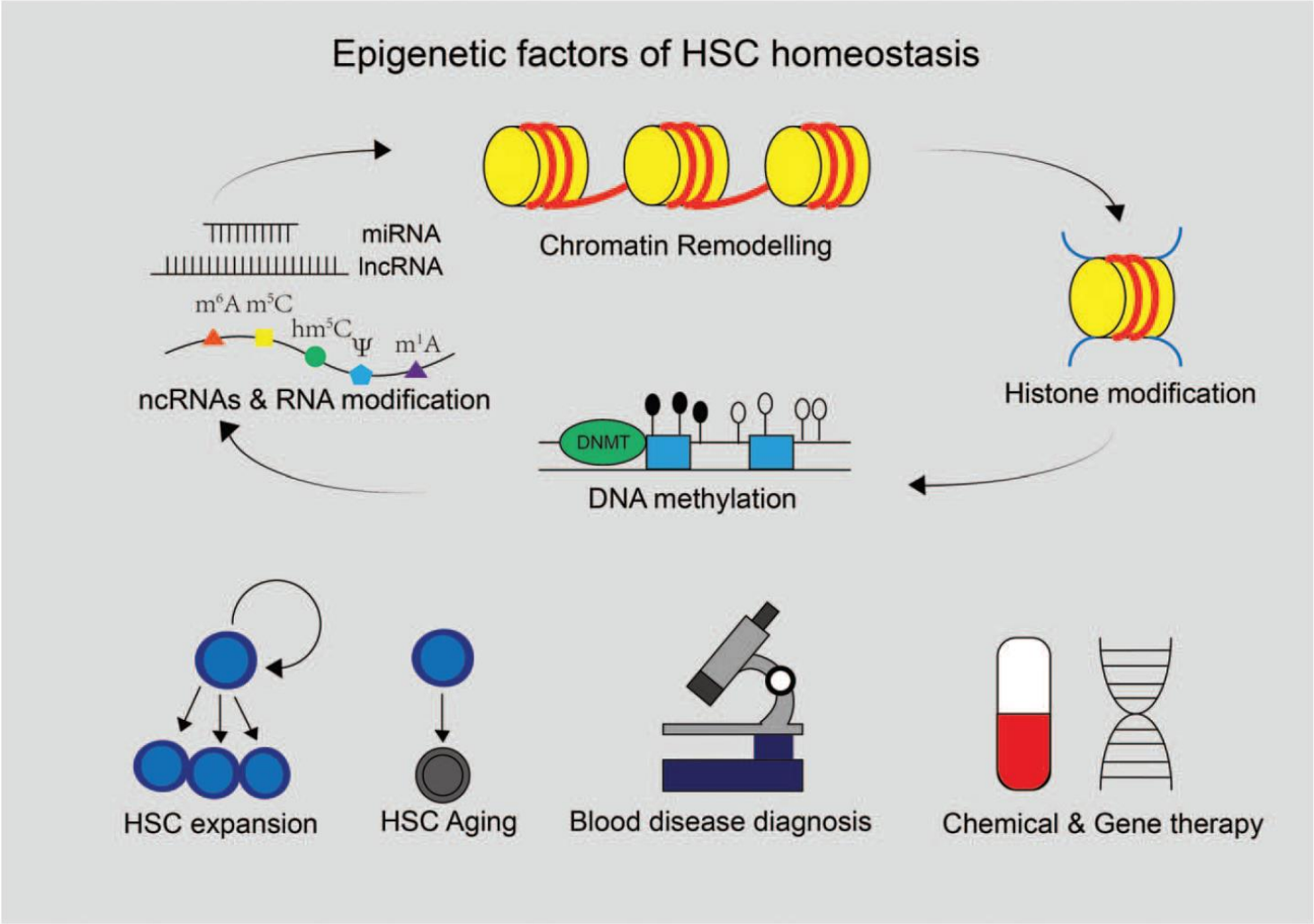


Figure 2. Epigenetic factors of HSC homeostasis and potential applications. Epigenetic factors including DNA methylation, histone modification, chromatir remodeling, non-coding RNAs, and RNA modification have been identified in recent decades. These factors are interactive and are essential for the regulation o HSC homeostasis. Understanding the regulatory mechanisms of these factors may unleash huge potential of HSCs in clinic such as blood disease diagnosis developing chemical and gene therapies through expanding HSCs or slowing down their aging.

Curr Stem Cell Rep. 2016 September ; 2(3): 183–196. doi:10.1007/s40778-016-0057-1.

MicroRNAs in Control of Stem Cells in Normal and Malignant Hematopoiesis

Table 1

The roles of specific miRNAs in HSC and LSC biology

miRNA	Species	Category	HSC/LSC	OE Phenotype	KD/KO Phenotype	Target
Let-7	Mouse	Self-renewal	HSC		Lin28 overexpression in adult HSCs leads to increased self-renewal	Hmga2
miR-101	Human/Mouse		HSC	OE bone marrow transplant, myeloid expansion hematopoietic expansion		TET2
miR-105	Human	Differentiation	HSC	OE CD34+ cells Enhanced megakaryocytes production		c-Myb
miR-106a	Human	Differentiation	HSC	OE progenitor cells proliferation increase, monocytic differentiation block	KD repressed proliferation and increased differentiation/maturation of monocytes	AML1
miR-99a/100~ 125b	Human/Mouse	Expansion	HSC	OE in HSC Expansion		TGFbeta Pathway
miR-125a miR-125b-2 miR-125b-2	Human/Mouse	Expansion Proliferation, Differentiation, Oncogenic Transformation, Self-renewal	HSC	OE in HSC Increases Colony Formation, increase in HSCs in vivo, Leukemia, myeloid biased proliferation, Increased proliferation and self-renewal/	Full-body miR-125a heterozygous KO leads to myeloproliferative phenotypes	Bak1, WT1, Lin28A, TET2, Bak1, Stat3, c-Jun, DICER1, ST18, PTPN7, PTPN18, PPP1R16A,
miR-126	Human/Mouse	Proliferation	LSC/HSC	OE in HSC Impaired cell-cycle entry, reduced hematopoietic contribution self -renewal OE in LSC quiescence, self renewal	KD HSC Enhanced self-renewal LSC Proliferation, differentiation	PI3K, AKT, GSK3beta pathway, HOXA9
miR-139-3p	Mouse/Human	Proliferation	HSC	OE in CD34+ cells strongly inhibited proliferation/cell cycle arrest and apoptosis	KD restored defective proliferation of Ercc1-deficient progenitors	HuR, EIF4G2
miR-142-3p	Zebrafish/Mouse/Xenopus	Development	HSC		KD Regulates HSC formation and differentiation	Irf7, TGFbeta signaling
miR-145	Human/Mouse					TIRAP
miR-146a	Zebrafish/Mouse/Human	Differentiation	HSC	OE bone marrow transplant transient myeloid expansion, decreased erythropoiesis, impaired lymphopoiesis.	KO decreased HSCs number, excessive myeloproliferation, HSC exhaustion, and hematopoietic neoplasms.	TRAF6, NFkappaB, IL-6
miR-150	Mouse	Engraftment Reconstitution	HSC	OE bone marrow transplant slowed recovery rates across major blood lineages after 5-fluorouracil treatment.	KO marrow recovers faster after 5-fluorouracil treatment.	C-Myb
miR-152	Human	Oncogenic Transformation	LSC			MLL and DNMT1
miR-155	Human/Mouse	Differentiation	HSC/LSC	OE bone marrow transplant blocked both erythroid and myeloid colony formation *in vitro.*	KO Resistance to experimental autoimmune encephalomyelitis	Pu.1, SHIP1
miR-15a	Human	Differentiation	LSC	OE CD34+ cells blocked both erythroid and myeloid colony formation *in vitro.*		C-Myb

MicroRNA, an Antisense RNA, in Sensing Myeloid Malignancies

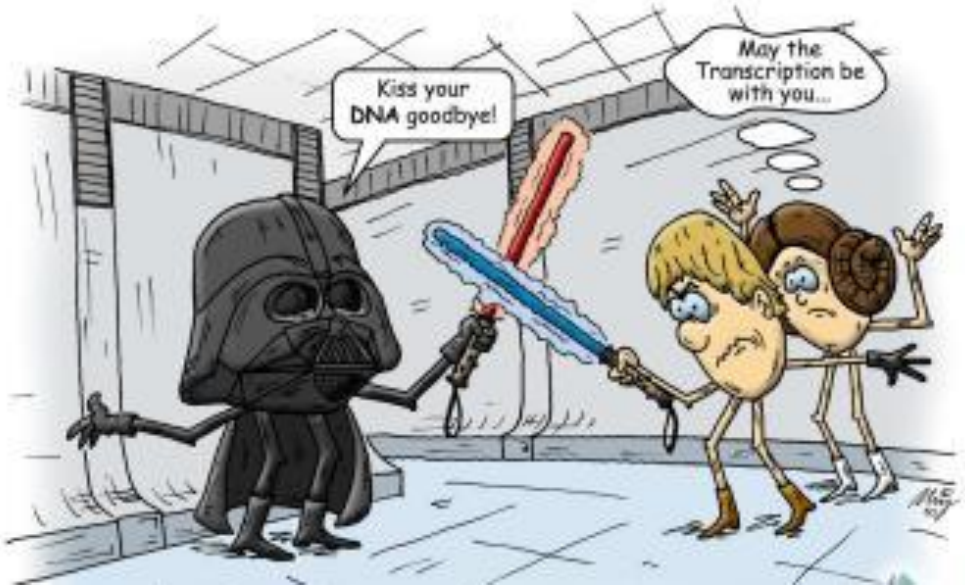
As the understanding of miRNA expression and action in myeloid malignancies continues to evolve, miRNAs have a great potential to serve as both the non-invasive biomarkers and a potential therapeutic target for leukemia. For example, miRNA expression signatures classify leukemias of uncertain lineage as either AML or acute lymphoblastic leukemia (98). miRNA expression profiles can also predict progression of MDS to AML (99) and survival outcome of AML patients (100, 101). Furthermore, circulating miRNAs have been recently demonstrated as an economical, non-invasive, and sensitive tool to monitor for minimal residual disease, which refers to the persistence of a small number of leukemic blasts in the bone marrow after chemotherapy and can ultimately cause disease relapse. Indeed, AML patients have a distinctive circulating miRNA expression profiles compared to healthy controls (102, 103), and an altered expression signature of serum miRNAs is observed after standard chemotherapy (104).

TABLE 1 | MicroRNAs (miRNAs) in myeloid malignancies.

miRNA	Expression profiles in leukemias	Reference
miR-22	High in AML/high in MDS	(12, 13)
miR-99	High in AML	(14)
miR-128a	High in AML	(15)
miR-155	High in AML	(16)
miR-182	High in AML	(17)
miR-221/miR-222	High in AML	(18)
miR-4262	High in AML	(19)
miR-29	Low in AML	(20)
miR-34a	Low in AML	(21)
miR-34b	Low in AML	(22)
miR-137	Low in AML	(23)
miR-142-3p	Low in AML	(24)
miR-194-5p	Low in AML	(25)
miR-204	Low in AML	(18)
miR-217	Low in AML	(26)
miR-223	Low in AML with t(8;21)	(18, 27–29)
miR-302a	Low in AML	(30)
miR-451	Low in AML	(31)
miR-650	Low in AML	(32)
miR-125b	High in AML	(33, 34)
miR-192	Low in AML	(35, 36)
miR-193	Low in AML	(37)
miR-124	Low in AML	(38, 39)
miR-181a	Low in AML	(18, 40)
miR-196b	High in AML	(34, 41)
miR-21	High in acute lymphoblastic leukemia, high in MDS	(42, 43)
miR-17–92/miR-20	High in CML/high in MDS	(44, 45)
miR-10a	Low in CML, high in MDS	(18, 46)
miR-126	High in AML	(47, 48)
miR-155	High in MDS	(46)
miR-130	High in MDS	(46)
MiR-144/451	Low in MDS	(12, 49)
miR-146a	Low in MDS	(46)
miR-150	Low in MDS	(46)
let-7a	Low in MDS	(46)

AML, acute myeloid leukemia; MDS, myelodysplastic syndromes.

Story goes on...



- More than 70% of all human miRNAs are located within regions of recurrent copy-number alterations in MDS and AML cell lines
- Ineffective erythropoiesis due to overexpression of miR-181, miR-221, miR-376b,miR-125b, miR-155
- Thrombocytopenia due to overexpression of miR-155, miR-126, miR-130
- High-risk MDS and decreased expression of miR-144/451

Good guy or bad guy: the opposing roles of microRNA 125b in cancer

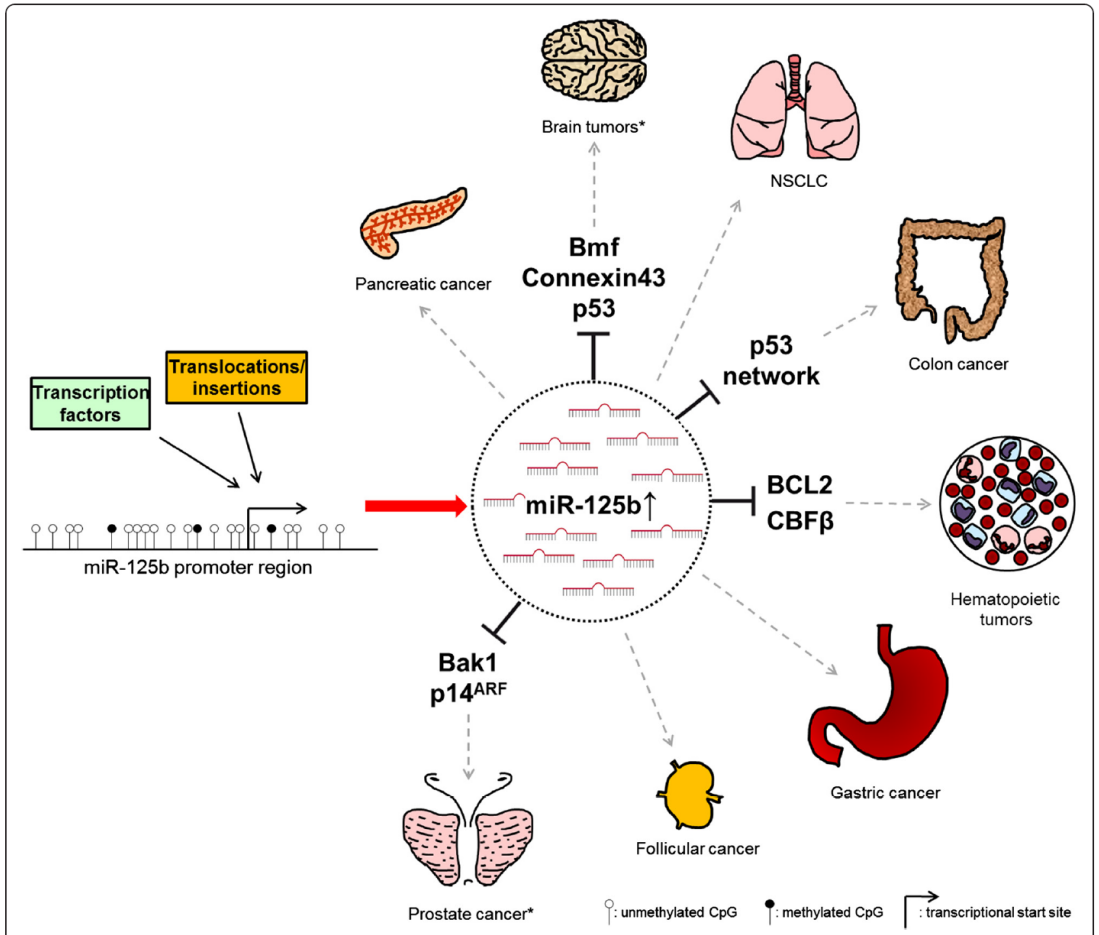


Figure 1 "The bad guy": miR-125b associated with oncogenic signaling in cancer. t (2;11) (p21, q23) translocations, miR-125b-1 insertions and androgen signaling have been shown to cause an upregulation of miR-125b. This blocks the translation of target mRNAs which might promote the formation of the indicated tumor types. See also Table 1. *Upregulation of miR-125b in brain tumors and prostate cancer is

miR-125b is a "double-edged" miRNA

Banzhaf-Strathmann and Edbauer *Cell Communication and Signaling* 2014, **12**:30
http://www.biosignaling.com/content/12/1/30

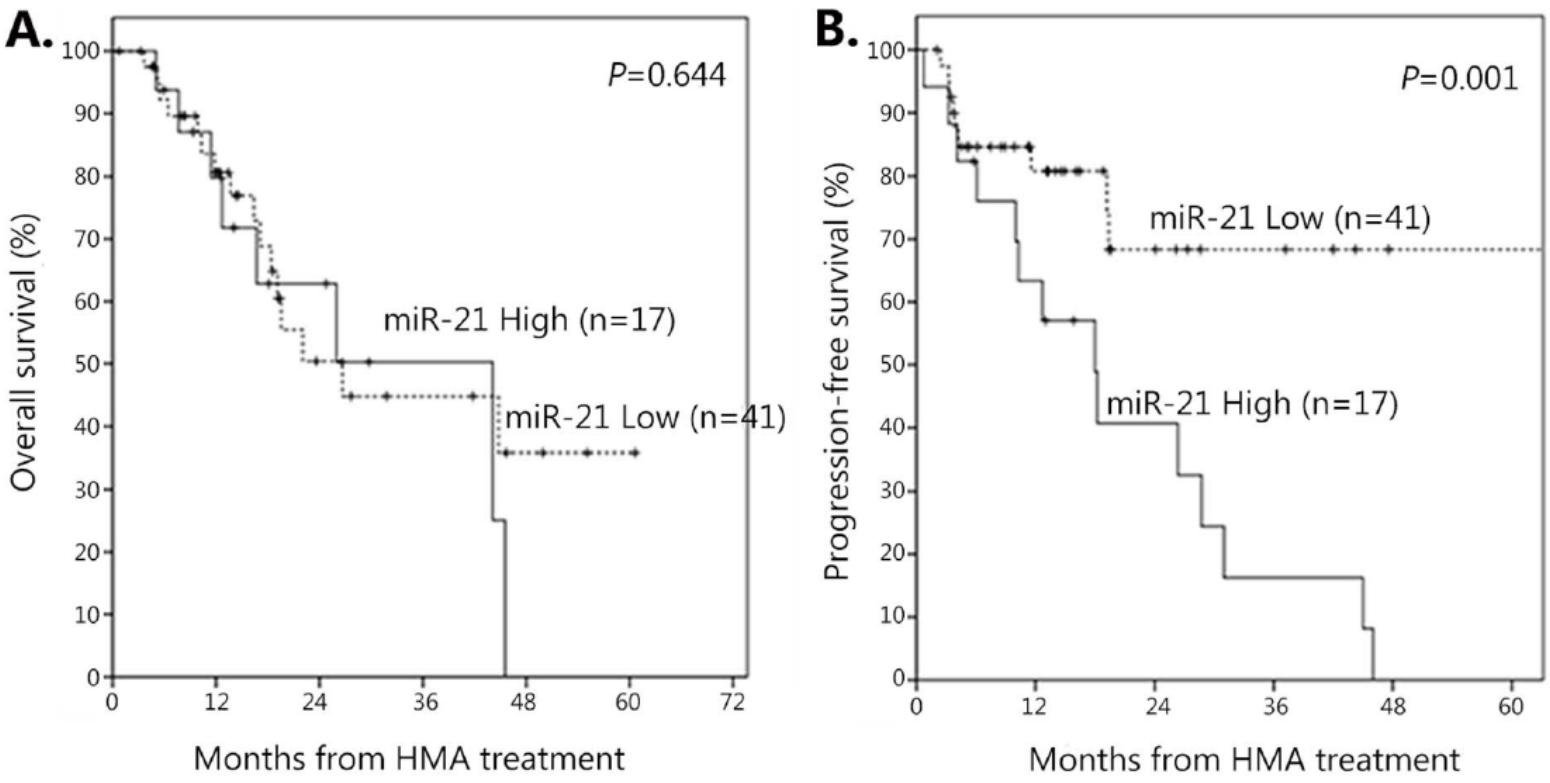
Plasma circulating-microRNA profiles are useful for assessing prognosis in patients with cytogenetically normal myelodysplastic syndromes

Table 6 Levels of plasma microRNAs comparing different patient subsets

	Healthy individual vs myelodysplastic syndromes	*Del(20q) vs other myelodysplastic syndromes*	*Del(7q)/−7 vs other myelodysplastic syndromes*	*Poor overall survival vs good overall survival*
hsa-miR-144	↓	↓	↑	↑
hsa-miR-206	↑	↑	—	—
hsa-miR-25	↓	—	↓	↑
hsa-miR-34b	↑	↑	—	—
hsa-miR-451	↓	↓	↑	↑
hsa-miR-493	↓	↑	—	—
hsa-miR-503	↑	↑	—	—
hsa-miR-651	↑	—	—	↓
hsa-miR-655	↑	—	—	↓
hsa-miR-92a	↓	↓	↑	—
hsa-miR-96	↓	—	↑	—

Symbols: ↑, upregulated; ↓, downregulated; —, no significant difference.

Serum microRNA-21 as a Potential Biomarker for Response to Hypomethylating Agents in Myelodysplastic Syndromes



Serum microRNA-21 as a Potential Biomarker for Response to Hypomethylating Agents in Myelodysplastic Syndromes

Table 2. Response to HMA therapy according to serum miR-21 level.

			Serum miR-21		
		Overall (n = 58)	Low (n = 41)	High (n = 17)	P-value
HMA cycles administrated (range)		6 (1–16)	7 (1–12)	6 (3–16)	0.474
HMA cycles for best response (range)		4 (2–14)	4 (2–12)	4 (2–14)	0.632
Time to response, months (range)		4.5 (1.0–50.5)	4.3 (1.0–50.5)	5.1 (1.0–23.8)	0.956
Response by IWG 2006 criteria, n (%)	CR/PR	4 (6.8)	3 (7.3)	1 (5.9)	0.542
HMA cycles administrated (range)	mCR	3 (5.2)	3 (7.3)	0	
	SD with HI	30 (51.7)	24 (58.5)	6 (35.3)	
	SD without HI	14 (24.1)	6 (14.6)	8 (47.1)	
	PD/Failure	7 (12.0)	5 (12.1)	2 (11.8)	
	CR/PR	4 (6.8)	3 (7.3)	1 (5.9)	
	mCR	3 (5.2)	3 (7.3)	0	
ORR (CR+PR+mCR+SD with HI)		37 (63.8)	30 (73.2)	7 (41.2)	0.012
Response duration, months (range)		39.1 (28.5–49.8)	47.2 (36.6–58.0)	18.1 (7.6–28.6)	0.067

MicroRNA profiles as predictive markers of response to azacitidine therapy in myelodysplastic syndromes and acute myeloid leukemia

Table 2
Univariate analysis for overall response rate and overall survival

Variable	n	ORR, n (%)	*P* value	OS, median (mo.)	*P* value
Age					
< 70 years	15	6 (40%)	0.930	19.6	0.385
⩾ 70 years	12	5 (42%)		21.2	
Sex					
Male	15	6 (40%)	0.930	19.6	0.837
Female	12	5 (42%)		24.9	
Blasts					
< 10%	7	3 (43%)	0.895	23.9	0.515
⩾ 10%	20	8 (40%)		19.4	
Karyotype					
Normal	8	3 (38%)	0.330	21.2	0.853
Isolated del(5q)	5	3 (56%)		25.9	
Other	13	3 (33%)		13.0	
Cytogenetics by IPSS-R					
Good	17	7 (41%)	0.334	25.9	**0.033**
Int/poor/very poor	9	2 (22%)		11.[illegible]	
Diagnosis					
MDS	19	8 (42%)	0.824	21.1	0.953
AML-MRC	8	3 (38%)		20.4	
miR-17-3p					
Low	15	3 (20%)	**0.014**	21.2	0.215
High	12	8 (67%)		19.1	
miR-100-5p					
Low	13	8 (62%)	**0.03[illegible]**	25.9	**0.047**
High	14	3 (21%)		16.4	
miR-133b					
Low	9	7 (78%)	**0.005**	19.1	0.915
High	18	4 (22%)		22.4	

Int – intermediate, ORR – overall response rate, OS – overall survival.

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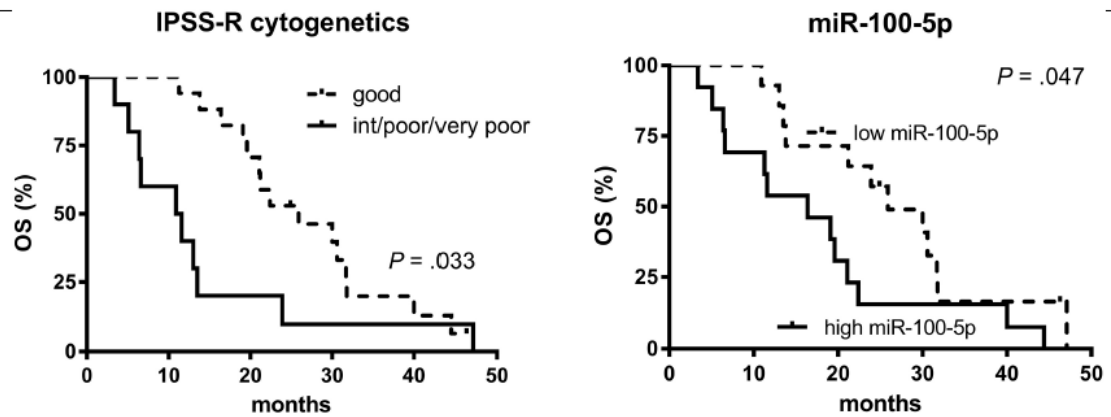

Fig. 4. Kaplan-Meier curves for the overall survival of patients stratified according to IPSS-R cytogenetics and miR-100-5p level.

Croatian MDS group

- To analyze 5-azacitidine response in HR-MDS by measuring miRNA let 7a, miRNA-21, miRNA-125a, miRNA-144,
- 1.before 5-AZA
- 2. after 4 cycles
- 3. after 6 cycles
- 4. at progression

Croatian Science Foundation

The **hsa-miRNA-125a** is involved in the process of myeloid leukemogenesis, and experimental studies have shown its impact on microRNA claster (**miRNA-125b, 99b, let7a**).

miR-125 a i b: inhibition of monocyte and granulocyte differentiation in myeloid neoplasms, apoptosis regulators

miR-126: upregulated in MDS low risk, erytropoiesis inhibitor

miR-99b: member of cluster miRNAs with miR-125 and miR-126

Gañán-Gómez, Irene & Wei, Yue & Pierce, Sherry & Bueso-Ramos, Carlos & Calin, George & Boyano Adánez, Menchu & García-Manero, Guillermo. (2014). Overexpression of miR-125a in Myelodysplastic Syndrome CD34+ Cells Modulates NF-κB Activation and Enhances Erythroid Differentiation Arrest. PloS one. 9

Curr Stem Cell Rep. 2016 September ; 2(3): 183–196. doi:10.1007/s40778-016-0057-1.

MicroRNAs in Control of Stem Cells in Normal and Malignant Hematopoiesis

MICRO RNAs (miRNAs) EXPRESSION LEVELS IN MYELODYSPLASTIC SYNDROME (MDS) – CROATIAN PILOT PROFILING

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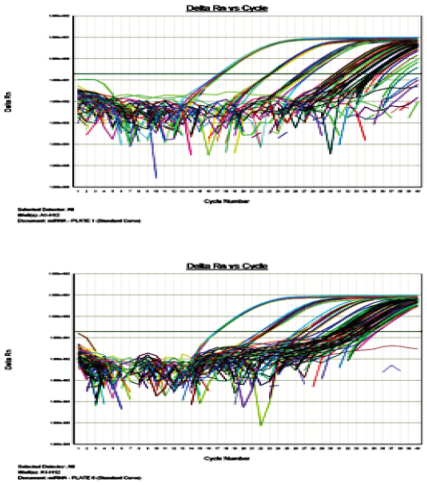
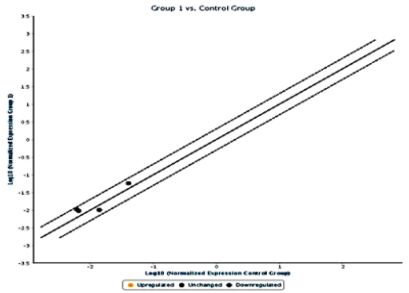
Department of Clinical Chemistry and Laboratory Medicine Merkur University Hospital, Zagreb, Croatia
Department of Internal Medicine Merkur University Hospital, Zagreb, Croatia

INTRODUCTION

Micro RNAs (miRNAs) as post- transcriptional regulators of gene expression are released by tumour cells into circulation. In biofluids such as plasma they were found at stable levels. Changes of specific circulating miRNAs expression levels observed in myelodysplastic syndrome (MDS) could represent new noninvasive molecular targets for detection and prognosis of MDS.



Pictures 1a- b. The baseline settings were the same across all qPCR runs to allow comparison of results.



MATERIALS AND METHODS

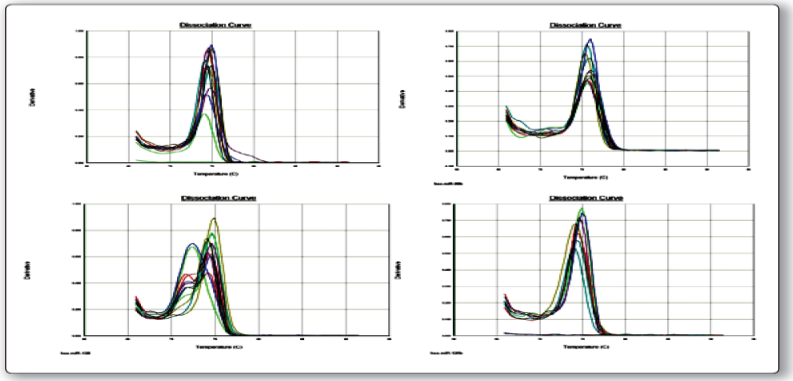
Materials and methods. miRNA profoling was done in a molecular laboratory accredited according ISO 15189. Whole blood from 4 healthy volunteers and 18 MDS patients diagnosed in Reference center for MDS Croatian Ministry of Health was drawn into EDTA containing tubes (BD Vacutainer), processed to plasma separation, miRNAs extra<ction (miRNeasy serum/Plasma Kit), reverse transcription (miScript II RT Kit) and qPCR measuring (miScript SYBR Green PCR Kit/Custom PCR Array). miRNA RT control assay (miRTC) and positive PCR control (PPC) were used to determine the presence of RT and PCR inhibitors. Data normalization (cell-miR-39-3p) and data analysis (web- based software) were done according to the manufacturer instructions (Qiagen).

AIM

To investigate the ability of qPCR measuring the plasma expression levels of four specific miRNAs: hsa-miR-125a-5p, hsa-miR-99b-5p, hsa-miR-126-3p, hsa-miR-125b-5p.

RESULTS

With the recommended Ct cut-off set at 35 PCR array reproducibility and RT efficiency were satisfactory. No statistically significant differences of miRNAs expression levels between healthy volunteers and MDS patients were observed. In MDS all miRNAs were up regulated, but not over 2-fold change of healthy volunteers. hsa-miR-125a was more than twice as high as miR-99b in MDS (1.72 vs. 0.72 fold change).



Pictures 2a-d. Specificity of qPCR runs. Specificity was assessed by the default software melting program immediately after the cycling program completion. Dissociation curves of four specific miRNAs with single peaks indicated presence of specific amplification products without primer dimers.

Table 1. qPCR data analyzed according the ΔΔCt method (web- based software).

Gene Symbol	AVG ΔCT		2^-ΔCT		Fold Change	Fold Up- or Down-Regulation	
	Group 1	Control Group	Group 1	Control Group	Group 1/ Control	Group 1/ Control	p-value
hsa-miR-125a	6.56	7.34	0.010615	0.006162	1.72	1.72	0.518396
hsa-miR-99b	6.72	7.24	0.009519	0.006638	1.43	1.43	0.262586
hsa-miR-126	4.12	4.61	0.057401	0.040950	1.40	1.40	0.402456
hsa-miR-125b	6.63	6.15	0.010089	0.014106	0.72	-1.40	0.122720
cel-miR-39	0.00	0.00	1.000000	1.000000	1.00	1.00	0.000000

The method was developed in collaboration with the IUC-T Oncopole, Laboratoire d`Anatomie Cytologie Pathologiques, Toulouse, France and and work supported by Ana Rukavina Fondation, Zagreb, Croatia.

CONCLUSION

In summary, we identified hsa-miR-125a as a potential noninvasive biomarker for MDS. Zuo et al. (2015) identified seven highly differentially expressed plasma miRNAs between good and poor survival groups of cytogenetically normal MDS patients. Therefore although this Croatian pilot profiling preliminary results are in concordance with the literature, extended profiling and some additional miRNAs such let-7a, hsa-miR-16, hsa-miR-144, hsa-miR-451,hsa-miR-651 or hsa-miR-655 in the future should identify real small changes in miRNA expression levels that could be useful for diagnostic purposes in MDS.

Literature: 1. Methods 50 (2010) 244-249.; 2. Clinical Chemistry 61:11 (2015) 1333- 1342

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microRNA analysis

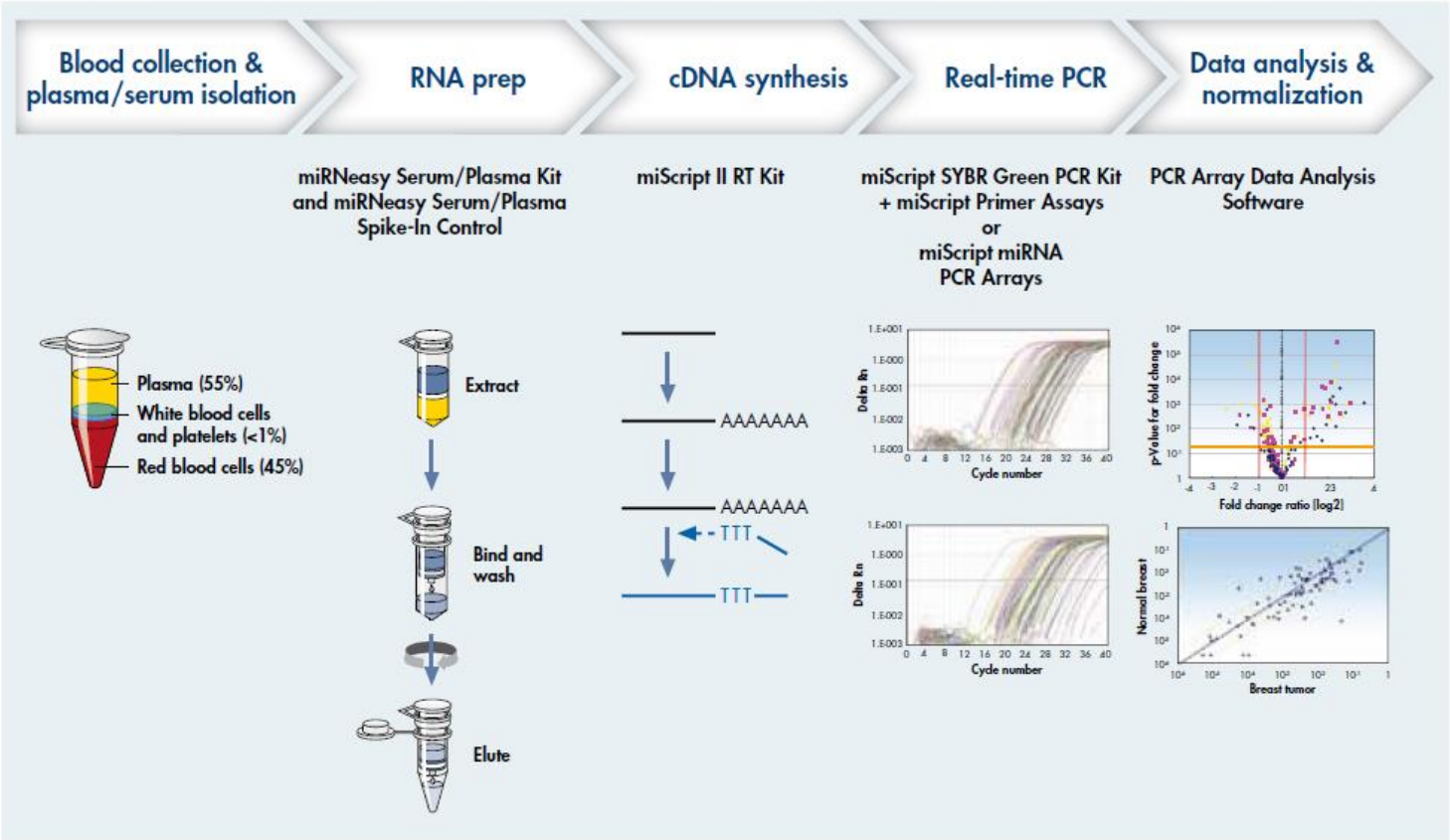

Figure 4. miScript PCR System workflow.

. Mestdagh, P. et al. (2009) A novel and universal method for microRNA RT-qPCR data normalization. Genome Biology 10, R64.

MICRO RNAs -99b-5p AND hsa-miR-125a-5p IN MYELODISPLASTIC SYNDROME

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BACKGROUND

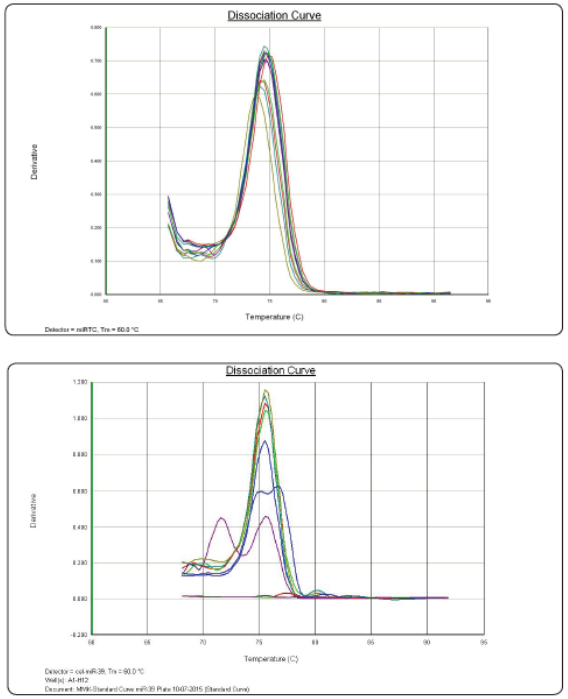
The pathogenesis of myelodisplastic syndrome (MDS) and the complex mechanism of disease transformation to more agressive acute myeloid leukemia (AML) has been objective of many experimental studies.
Due to molecular mechanisms investigated micro RNA (miRNA) profiling of specific circulating micro RNAs (miRNAs) could represent new noninvasive molecular target for detection and prognosis of MDS.
Its clinical effectiveness is likely to be affected by various methodological issues and qPCR platform used.
The aim was to investigate the plasma expression levels of two miRNAs (hsa-miR-99b-5p and hsa-miR125a-5p) specific for MDS.

METHODS

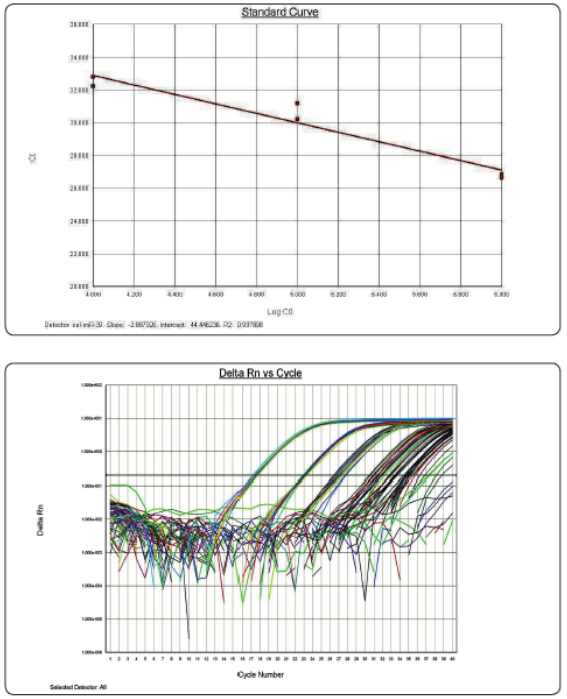
The plasma expression levels of hsa-miR-99b-5p and hsa-miR-125a-5p were investigated in a molecular laboratory accredited according ISO 15189. Whole blood from 4 healthy volunteers and 33 MDS patients diagnosed in Reference center for MDS Croatian Ministry of Health was drawn into EDTA containing tubes and processed to plasma separation, mirNAs extraction (miRNeasy Serum7plasma Kit), reverse transcription (miScript II RT Kit) and qPCR measuring (miScript SYBR Green PCR Kit/Custom PCR Array). For the determination of the presence of RT and PCR inhibitors miRNA RT control assay (miRTC) and positive PCR control (PPC). Data normalization (cell miR-39-3p) and data analysis (web- based software) were done according to the manufacturer instructions (Qiagen).

MICRO RNAs -99b-5p AND hsa-miR-125a-5p IN MYELODISPLASTIC SYNDROME

ZAKLADA **ANA RUKAVINA**



Pictures 1a - b. Specificity of qPCR runs. Dissociation curves of controls (miRTC, cell-miR-39) and specific miRNAs (hsa-miR-99b, hsa-miR-125a) with single peaks indicated presence of specific amplification products without primer dimers or PCR inhibitors.



Pictures 2a - b. The baseline settings were the same across all qPCR runs in the same analysis to allow comparison of qPCR data (analyzed according ΔΔ Ct method). Cell-miR-39 standard curve showed satisfactory qPCR efficiency (calculated from the slope -2,887).

Table 1. qPCR data analyzed according ΔΔ Ct method. (web- based software) .

miRNAs	AVG Δ C_T		2^-Δ C_T		Fold Change	Fold Up- or Down Regulation	
	MDS	CTRL GROUP	MDS	CTRL GROUP	MDS / CTRL	MDS / CTRL	p- value
hsa-miR-125a	6.14	7.37	0.0141	0.0061	2.30	2.30	0.3978
hsa-miR-99b	6.31	7.24	0.0125	0.0066	1.90	1.90	0.1336
cell-miR-39	0.00	0.00	1.0000	1.0000	1.00	1.00	0.0000

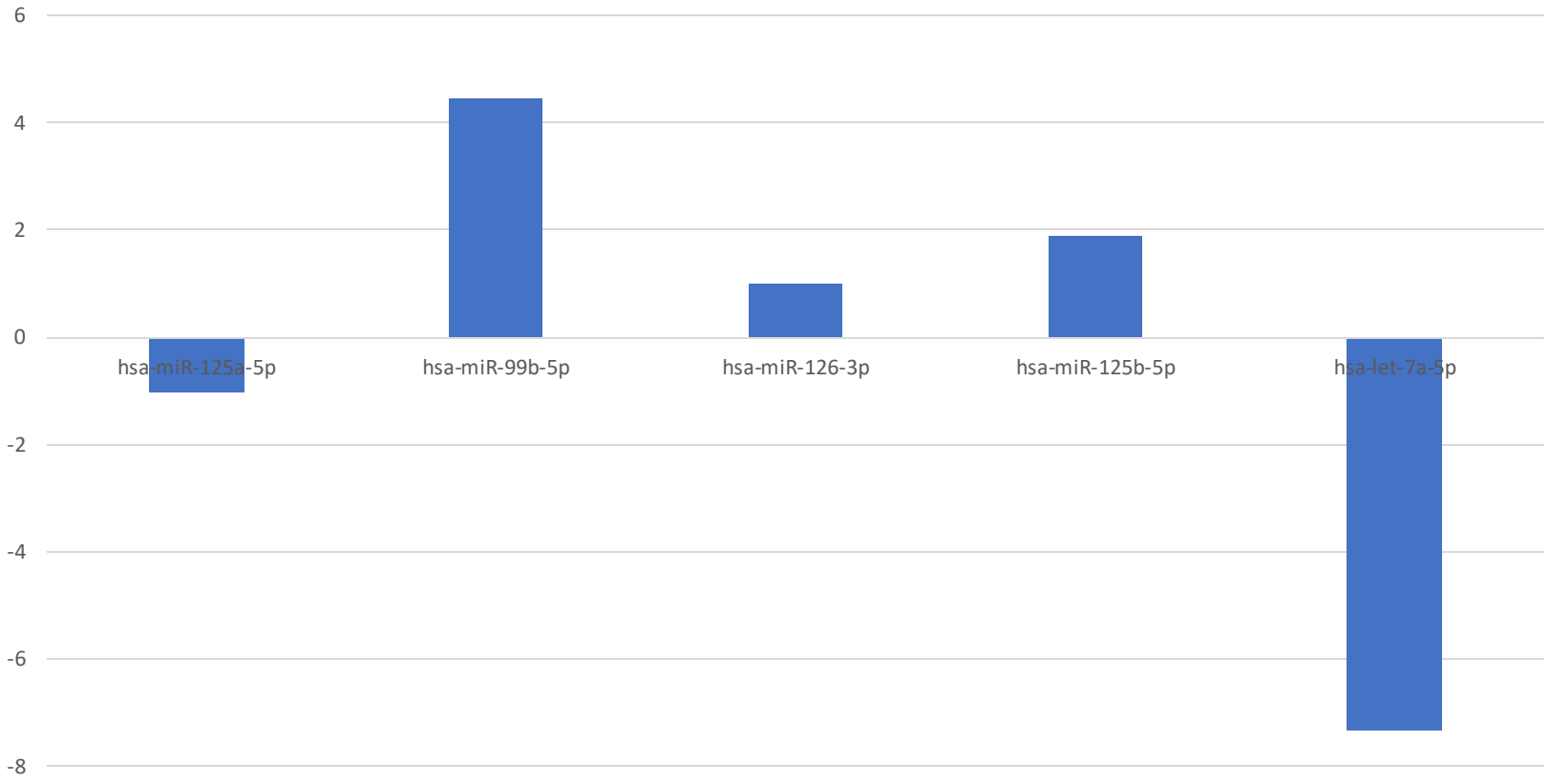
Patient characteristics

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
						25th	50th (Median)	75th
RBC	41	3,11	0,54	2,07	4,79	2,80	3,06	3,25
Hgb	41	102,51	15,95	75,00	143,00	92,50	101,00	112,00
MCV	41	98,60	8,97	77,50	115,00	91,90	99,00	105,00
Rtcdg	41	47,30	56,74	5,50	351,20	21,35	39,00	46,40
WBC	41	4,65	2,71	0,69	13,60	2,84	4,00	6,76
ANC	41	2,63	1,95	0,13	9,20	1,08	2,16	3,80
ly	41	1,40	0,66	0,30	2,60	0,92	1,30	1,96
Plt	41	166,15	115,19	6,00	427,00	83,00	145,00	257,00
LDH	41	190,15	75,59	97,00	546,00	153,50	176,00	195,50
CRP	41	8,75	12,75	0,20	61,60	1,30	3,50	11,50
ESR	41	34,78	33,12	2,00	120,00	11,50	20,00	46,00
Age at dg	41	68,83	9,97	31,00	84,00	63,00	70,00	76,00
IPSS score	41	0,49	0,68	0,00	3,00	0,00	0,50	0,50
IPSS-R score	41	2,44	1,96	0,00	9,50	1,05	2,00	3,70
WPSS score	41	1,60	1,51	0,00	6,00	0,00	1,50	2,50

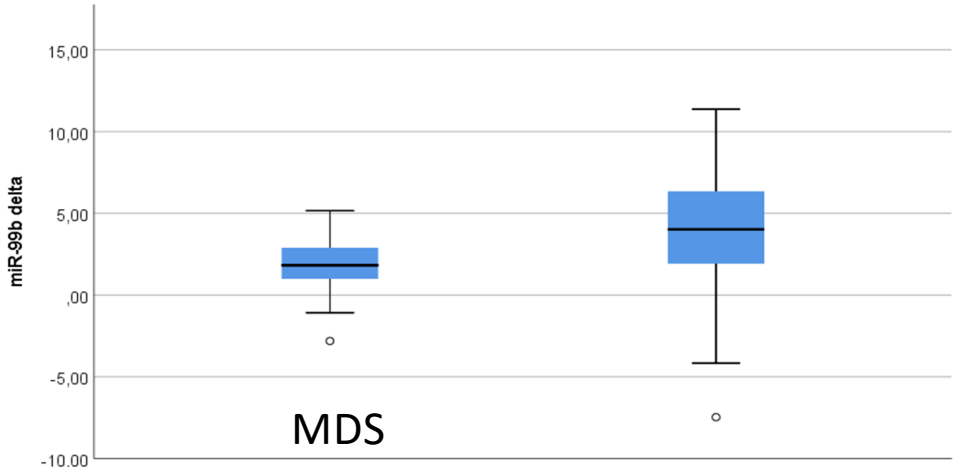
Patologic karyotype	no	33	80,5%
	yes	8	19,5%
IPSS	LOW	18	43,9%
	INT1	18	43,9%
	INT2	4	9,8%
	HIGH	1	2,4%
IPSS-R_gr	VERY LOW	17	41,5%
	LOW	12	29,3%
	INT	5	12,2%
	HIGH	5	12,2%
	VERY HIGH	2	4,9%
WPSS	VERY LOW	12	29,3%
	LOW	9	22,0%
	INT	10	24,4%
	HIGH	8	19,5%
	VERY HIGH	2	4,9%

miRNA expression level in MDS



miR-99b – diagnostic parameter

		N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
							25th	50th (Median)	75th
miR-125a delta	MDS	41	1,798	1,284	-0,731	4,449	1,026	1,716	2,462
	Control	26	1,863	4,495	-7,545	9,834	0,674	2,034	4,279
miR-99b delta	MDS	41	1,811	1,627	-2,812	5,163	0,883	1,824	2,962
	Control	26	3,894	4,398	-7,472	11,373	1,656	4,023	6,361
miR-125b delta	MDS	41	2,442	0,891	1,096	4,918	1,724	2,427	3,174
	Control	26	3,279	3,902	-8,861	11,083	1,057	2,937	5,424
miR-let7a delta	MDS	10	-0,834	0,891	-2,972	0,502	-1,034	-0,751	-0,360
	Control	22	0,524	3,558	-3,991	8,443	-2,094	-0,401	1,861



How to improve diagnostic/prognostic value of miRNA?

- Well-designed and controlled multicenter studies of the same disease to avoid substantial variations and inconsistency in results
- Recruite much greater number of participants that allow assembling them better according to sex, specific stage and length of disease and minimize the influence of uncontrollable confounding factors
- Better optimization and standardization procedure of collecting and handling biological samples
- Integrative analysis of circulating miRNAs with already verified and established diagnostic markers

Circulating miRNAs: new targets in myelodysplastic syndromes

Authors: Inga Mandac Rogulj &
Slobodanka Ostojić Kolonić

Clearly, a progress has been made in our understanding of miRNAs impact of pathogenesis of MDS, but more extensive research with several paradigms shifts is needed to clarify the role of this complex mechanism in the genetic and epigenetic MDS setting. It would also be of great interest to see what miRNAs are potential therapeutic or disease modifying targets.

What will MDS future bring?

That is the question...

But the truth is...

THE
FUTURE
DEPENDS ON WHAT YOU DO
TODAY
Mahatma Gandhi

Thank You
תוֹדָה
Toe-dah

Croatian MDS Team