

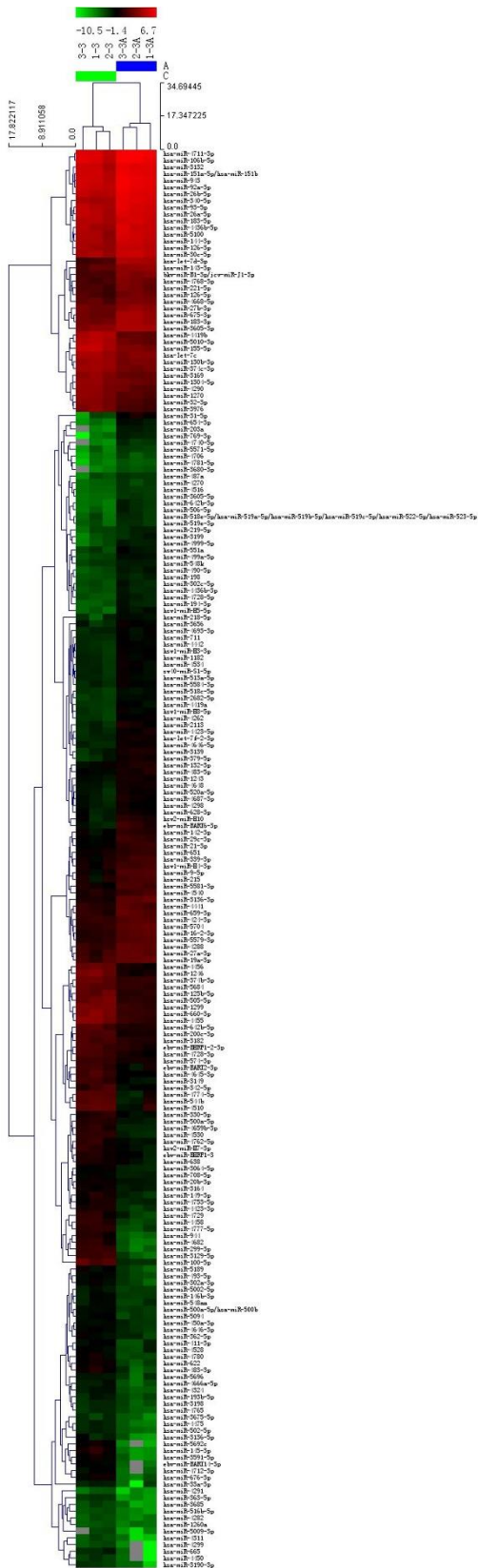
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# MicroRNAs Dysregulation Associated with Red Blood Cell Storage

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## Supplemental Material



Supplementary Figure 1. Column 3-3,1-3,2-3 represents three fresh samples, and 3-3A,2-3A,1-3A represents three 20 d stored samples. Red color represent high level expression, green color represent low level expression.

Supplementary Table 1. qRT-PCR primers used for miRNAs expression

hsa-miR-203a (5'-3')	GUGAAAUGUUUAGGACCACUAG
hsa-miR-654-3p	UAUGUCUGCUGACCAUCACCUU
hsa-miR-31-5p	AGGCAAGAUGCUGGCAUAGCU
hsa-miR-769-3p	CUGGGAUCUCCGGGGUCUUGGUU
hsa-miR-96-5P	UUUGGCACUAGCACAUUUUUGCU
hsa-miR-150-5P	UCUCCCAACCCUUGUACCAGUG
hsa-miR-196a-5p	UAGGUAGUUUCAUGUUGUUGGG
hsa-miR-197-3p	UUCACCACCUUCUCCACCCAGC

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Supplementary Table 2. qRT-PCR forward and reverse primers used for gene expression.

GENE	DIRECTION	PRIMER
<i>GAPDH</i>	Forward	5'-CAGTAGAGGCAGGGATGATGTTCT-3'
	Reverse	5'-CATGGGTGTGAACCATGAGAAGTA-3'
<i>RICTOR</i>	Forward	5'-GGAAGCCTGTTGATGGTGAT-3'
	reverse	5'-GGCAGCCTGTTTTATGGTGT-3'
<i>ATM</i>	Forward	5'-ACTATCCCAATACACTGCTGGAGA-3'
	Reverse	5'-TTTGAGCAACTGACTGGCAAAC-3'
<i>CASP10</i>	Forward	5'-TCATTGTCAACAACCACAGCTTTAC-3'
	Reverse	5'-AGGACCATCTCCATTTCCACTTTC-3'
<i>CASP8</i>	Forward	5'-GAACCTGGTACATCCAGTCACTTTG-3'
	Reverse	5'-AGTTCACTTCAGTCAGGATGGTGAG-3'
<i>IL3</i>	Forward	5'-AACACACTTAAAGCAGCCACCTTTG-3'
	Reverse	5'-AAACTCTTGACAGCCCTGTTGAATG-3'
<i>BIRC6</i>	Forward	5'-GATGGAAACATTTCGACAAGCAAC-3'
	Reverse	5'-AGTCCTGCCTACCCGCTTATCAC-3'
<i>MAP3K14</i>	Forward	5'-GTCTGGAATACCTCCACTCACGAA-3'
	Reverse	5'-AGGCCATCAGGTTGAAGACACA-3'
<i>EPB41LAB</i>	Forward	5'-GAAACAGCTGTGGAATTAGCTGCTC-3'
	Reverse	5'-CAAGCTCTGGTGTGTGTTCTGGA-3'
<i>BTG2</i>	Forward	5'-CGTGAGCGAGCAGAGGCTTA-3'
	Reverse	5'-TGTGGTTGATGCGAATGCAG-3'

Supplementary Table 3. Screened miRNAs and their target mRNAs.

miRNA	Apoptosis-related			RBC-related	Senescence-related	
miR-654-3p	CASP10 Δ	P2RX4(2)	BIRC6	-	CDKN2A (2)	MAPK14
	MyD88 Δ	FRMPD4	CHUK		ID1 (2)	CHEK1
	<b>ATM</b> Δ	COL3A1	AKT3		IGFBP5	ETS2
	PPP3CA Δ	CASP2	SON		<b>ATM</b>	
	RICTOR (2)	GRIK2	NF1			
	MAPK1IP1L (2)					
miR-31-5p	TRADD (2) Δ	E2F6 (2)	BTG2	EPB41L4B	CALR (2)	SPARC
	CASP10 Δ	IGFBP3	IKBIK		CDKN1B (2)	BMI1
	<b>PRKAR1B</b> (2)	SEPT11	EGR1		IGFBP7 (2)	ETS2
	PPP3CA Δ	CASP2	NF1		CREG1 (2)	
	SEPT9 (2)	IKBKB	LTA			
	MAP3K14 (3) Δ					
miR-196a-5p	CASP8Δ (2)	CHUK (2)	FAS Δ	EPB41L4B	CDKN1B (2)	IGFBP3
	BIRC6 (3)	<b>ABL1</b> (2)	CASP3 Δ		COL3A1 (2)	CHEK1
	RICTOR (2)	PRKAR1A	ING5(2)		COL1A1(2)	CCNA2
	CFLAR (2)	FRMPD4	AKT3		<b>ABL1</b> (2)	
	NFKBIA (2)	PPP3R1				
miR-203a	CASP10 Δ	BIRC5 (2)	LTA (2)	EPB41L4B	CITED2 (3)	RB1 (2)
	CASP8 Δ	BIRC2 (2)	SON (2)		BMI1 (3)	MAPK14
	<b>ATM</b> (2) Δ	FARP1 (2)	AKT3 (2)		IGFBP5 (2)	COL3A1
	IL3 (2) Δ	AKT2 (2)	IKBIK (2)		E2F3 (2)	CCNA2
	CASP3 Δ	ESR1 (2)	BIRC6		ETS2 (2)	CCND1
	MyD88 Δ	BAG3 (2)	EGR2		CALR (2)	CREG1
	BCL2 Δ	DFFB (2)	IRAK2		SPARC (2)	<b>ABL1</b> (2)
	FAS Δ	<b>ABL1</b> (2)	CIDEB		TBX3 (2)	<b>ATM</b> (2)

	PPP3CA Δ	<b>MAP2K1</b>	GRIK2		GLB1 (2)	EGR1
	PIK3CA (2)	RICTOR	CASP2		<b>MAP2K1</b>	ING1
	IKBKB (2)	PRKAR1A	BNIP3		<b>PIK3CA</b> (3)	
	BCL2L2 (2)	SEPT11	P2RX4			
	PIK3CD (2)	PPP3R1	CHUK			
	DIABLO (2)	MAK11P1L	NF1			
miR-197-3p	CASP8 Δ	IKBKB(2)	CFLAR	EPB41L3	IGFBP3 (2)	IGFBP5
	CASP3 Δ	<b>MAP2K1</b>	BIRC5		CHEK1 (2)	CREG1
	CASP7 Δ	SEPT11	BIRC2		E2F1 (2)	EGR1
	PPP3CA Δ	RICTOR	GRIK2		COL3A1	RB1
	ENDO G (2) Δ	PPP3R1	NF1		<b>MAP2K1</b>	
	ENDO G (2)	IRAK2	SON			
	IKBIK (2)	CHUK				
miR-150-5p	CAS10 (2) Δ	TRAF2 Δ	AKT3 (2)	EPB41L3	CDKN1B (2)	CCNA2
	CASP8 Δ	TP53 (3)	RICTOR		SPARC (2)	ETS2
	CASP6 Δ	IRAK2(3)	PIK3R1		EGR1 (2)	E2F3
	PRKAR1A(2)	EGR2 (3)	NF1		MAPK14	TBX3
	MAPK1IP1L	CFLAR (2)				
miR-769-3p	CFLAR (2)	NOL3 (2)	CHUK	-	SOD2 (2)	MAPK14
	CIDEB (2)	PIK3R5 (2)	BMT1		COL3A1	BMI1
	FRMPD4 (2)	P2RX4	TP53			
	PRKAR1A					
miR-96-5p	CASP8 Δ	RICTOR (2)	<b>IGF1R</b>	EPB41L4B	CCND1 (2)	FN1 (2)
	CASP9 (2) Δ	SEPT11 (2)	<b>ABL1</b>	EPB41L3 (2)	GLB1 (2)	ETS2
	BCL2 Δ	PPP3R1 (2)	AATK (2)		MAPK14(0)	RB1
	PPP3CA Δ	PIK3R1 (2)	NF1 (2)		<b>MAP2K1</b> (3)	<b>IGF1R</b>
	PROK2 (3)	FARP1 (2)	SEPT9		<b>MAP2K3</b> (2)	<b>ABL1</b>
	AIFM3 (3)	CASP2 (2)	IKBKB			
	<b>MAP2K1</b> (3)	P2RX4 (2)	IKBIK			

	PRKAR1A (2)	RGS2 (2)	GRIK2			
	MAPK1IP1L (2)	NOD1 (2)	BIRC6			
	FRMPD4 (2)	DOK4 (2)	BIRC2			
	<b>MAP2K3</b> (2)	CTSB (2)	SON			

$\Delta$  mRNAs belonging to apoptosis pathway map in *KEGG*. Words in **bold** indicate that this mRNA was targeted by two miRNAs. The number between brackets indicates the number of predicting software among the three software programs used.

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**Supplementary Table 4.** Target mRNAs expression in stored RBCs group.

Relative mRNA expression			
mRNA	Fresh RBCs	20-day storage RBCs	<i>p</i> -value
BTG2	1.10±0.81	2.13±0.79	0.150
BIRC6	1.80±1.28	0.55 ±0.26	0.389
RICTOR	2.12±1.79	0.45±0.29	0.047
CASP8	2.08±0.66	0.38±0.16	0.042
EPB41L4B	1.23 ±0.82	0.44 ± 0.35	0.244
CASP10	1.97±1.81	0.36±0.29	0.025
IL-3	1.29 ±1.02	0.68± 0.24	0.267
ATM	1.15± 0.66	0.37 ±0.35	0.162
MAP3k14	1.13 ±0.47	0.44 ±0.31	0.209