

Supplementary material for Erdogan B, et al., Methylation of promoters of microRNAs and their host genes in myelodysplastic syndromes, *Leukemia & Lymphoma*, 2013; doi: 10.3109/10428194.2013.790542.

Supplementary Table 1. Patient characteristics for samples used in the pyrosequencing studies.

Age	Gender	Patient Set	Patient Diagnosis	Treatment Status
76	F	N.C	joint replacement	
60	M	N.C	joint replacement	
51	F	N.C	joint replacement	
63	F	N.C	joint replacement	
80	M	N.C	h/o new dx FL	
59	F	N.C	PRIOR FL	
12	M	N.C	NEW DX B CELL LYMPHOMA	
65	F	N.C	RELAPSED CUT DLBCL	
64	F	N.C	DLBCL IN 1998	
14	F	N.C	RHABDOMYOSARCOMA	
49	F	N.C	RELAPSED FL	
64	M	N.C	PRIOR FL	
57	F	N.C	NEW DX BCL	
62	M	N.C	DLBCL	
38	F	N.C	NEW DX DLBCL	
38	M	N.C	relapsed cHL	
21	M	N.C	NEW LARGE BCL OF MEDIASTINUM	
33	F	N.C	new NSHL	
60	M	N.C	new dx CNS DLBCL	
72	M	N.C	new dx intraventricular mass	
39	F	N.C	staging for follicular lymphoma	
72	M	N.C	s/p SCT, now relapsed mantle cell lymphoma	
52.23	average			
19.49	stdev			
	45% M			
63	F	MDS	RCMD	growth factors
72	M	MDS	RCMD	untreated
74	F	MDS	RCMD	untreated
69	M	MDS	RCMD	untreated
78	F	MDS	RCMD	untreated
66	F	MDS	RA	untreated
78	F	MDS	RA	growth factors
73	M	MDS	RA	immunosuppressives
76	M	MDS	RAEB-2	chemotherapy
59	M	MDS	RCMD	azacytidine 9 months prior
69	M	MDS	RAEB-2	thalidomide
47	M	MDS	RCMD	chemotherapy
50	F	MDS	RCMD	chemotherapy
69	M	MDS	RCMD	azacytidine 12 months prior
56	F	MDS	RA	growth factors
58	M	MDS	RCMD	azacytidine 24 months prior, followed by chemotherapy and cord blood SCT
73	M	MDS	RA	untreated
51	M	MDS	RARS	immunosuppressives
87	M	MDS	RCMD	growth factors
60	M	MDS	RCMD	thalidomide 18 months ago x 1y, decitabine 6 months prior, then panobinostat x 3 months
66.4	average		60% RCMD (12)	
10.64	stdev		also includes 5 RA, 1 RARS, 2 RAEB2	
	65% M			
66	M	MDS	RA	started on decitabine x 4 cycles, now vidaza x 1 cycle
58	M	MDS	RCMD	on azacytidine for 2 years, stable
57	M	MDS	RAEB-2	s/p 4c azacytidine no response
79	M	MDS	RAEB-2	on decitabine x 3 cycles with no response
56	M	MDS	RAEB-1	azacytidine x 5 cycles, then enocitabine x 2months
78	M	MDS	RCMD	s/p azacytidine x 8 cycles with initial response, now worsening anemia due to renal failure (polyclonal plasmacytosis)
65.67	average		1 RA, 2 RCMD, 1 RAEB1, 2 RAEB2	
10.56	stdev			
	100% M			

Supplementary Table 2. Primers used in pyrosequencing (all sequences listed from 5' to 3').

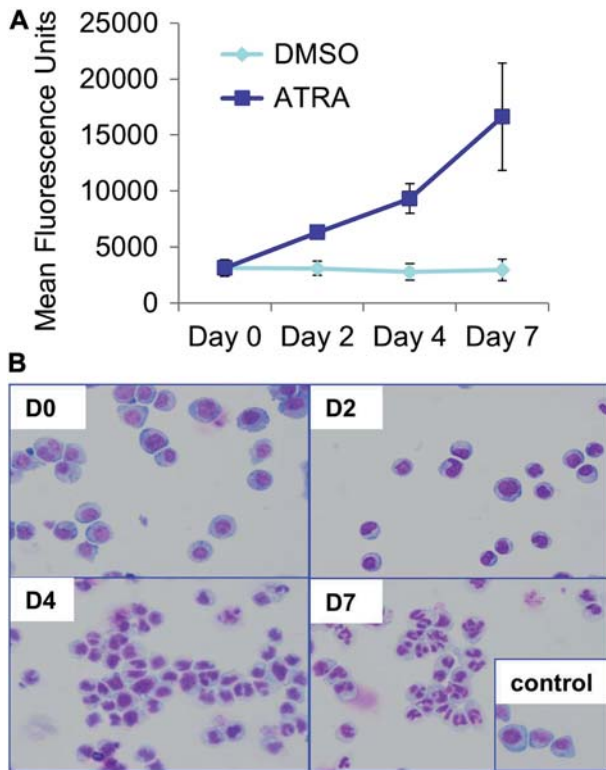
Host Gene Name	Forward Primer	Reverse Primer	Sequencing Primer	Amplicon Length [bp]
BIRC6	GTAAGGGAGGGGGTTTTTATT	Bio-CCAACCTCCCAATTTCTTCCTAT	GGTTAATTTTTTTTTTTTGT	127
TECR	GGATGTTGTTTGTGTTATTTAAT	Bio-CCACCAATCCAAAAAATCTCTAC	AATCCAAAAAATCTCTAC	142
IGF2	Bio-GGGATTTGGTTGAGGTTTTAA	CCATCCCCAATTTCTAAAATACC	TTTTTTTGTGGGGTAG	275
IGF2-DMRO	AGGGGGTTTATTTTTTAGGAAGTA	Bio-AACAAAAACCACTAACACACAACCTA	GGGTTTATTTTTTAGGAAGTAT	85
PANK3	GTTAGGTTTGAGGGGAGGAAT	Bio-CAACCCCTCTTACAAAATTTCT	GGTTTGAGGGGAGGA	164
WWP2	Bio-TGGTTAGTATTGAGAGTTTG GTTAGTTT	CCCAACTCCTCTCTACTTCC	TTAGTTTTTTAGGGTTAGG	112
EVL1	Bio-GAGGGAGGTGGGGTGAATAGT	AAACCCCCACCCCTACCATCT	ACCCCTACCATCTTA	284
RPS11	Bio-GGGAAGATGGAGGATATTT AGGT	CCCCTCCACTCCAATTAATCTC	ATTAATCTCTAAAAATAAT	167
PPARGC1B	QIAGEN Pyromark CpG Assay Hs_hsa-mir-378_01_PM Cat #PM00022519			176
ZNF207	QIAGEN Pyromark CpG Assay Hs_ ZNF207_01_PM Cat #PM00177688			212
SRSF2	QIAGEN Pyromark CpG Assay Hs_ MFSD11_02_PM Cat#PM00065359			180
SFRP2	QIAGEN Pyromark CpG Assay Hs_ SFRP2_01_PM Cat#PM00018802			110

Supplementary Table 3. Expression changes of miRNAs and their host genes during an assay of myeloid maturation (HL60/ATRA).

miRNAs	Fold increase	Host Genes	Fold increase
miR-103	1.22	PANK3	1.50
miR-140	1.30	WWP2	1.52
miR-150	1.01	RPS11	1.28
miR-342	1.13	EVL	1.46
miR-378	-0.83	PPARGC1B	1.18
miR-483	1.20	IGF2	not available
miR-632	1.09	ZNF207	1.25
miR-636	1.21	SFRS2	1.30

Supplementary Table 4. Compiled methylation results (as percent methylation) for methylation regions examined by pyrosequencing in promoters of WWP2, PPARGC1B, IGF2, ZNF207, and SFRS2. P values obtained from two-tailed t-test analysis of MDS samples (untreated or treated) compared to normal controls (NC).

	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7
SRSF2							
NC (22)							
average	1.318	2.091	1.682	4.455	2.909		
stdev	0.568	0.921	1.729	6.486	4.363		
MDS (20)							
average	3.100	6.050	3.450	11.450	5.500		
stdev	4.447	8.599	5.000	21.010	10.605		
P value vs NC	0.091	0.054	0.147	0.167	0.319		
MDS post Rx (6)							
average	1.833	2.333	1.667	4.500	2.833		
stdev	0.753	1.033	0.816	2.074	1.722		
P value vs NC	0.165	0.618	0.976	0.978	0.949		
ZNF207							
NC (22)							
average	13.227	7.545	5.591	8.500	12.682		
stdev	8.772	4.964	4.067	6.292	8.632		
MDS (20)							
average	24.200	14.250	11.900	15.350	24.500		
stdev	17.356	10.290	9.239	11.926	19.720		
P value	0.017	0.013	0.009	0.029	0.020		
MDS post Rx (6)							
average	9.667	5.667	5.167	5.833	9.833		
stdev	2.658	1.366	2.483	1.835	1.941		
P value	0.112	0.128	0.755	0.095	0.167		
IGF2							
NC (32)							
average	7.156	3.156	2.469	5.469	4.531	34.625	97.156
stdev	9.381	2.477	4.040	12.914	14.675	4.654	6.811
MDS (20)							
average	21.471	2.706	1.000	1.824	1.706	33.000	90.950
stdev	31.814	1.359	0.612	1.286	1.263	8.681	15.856
P value	0.087	0.415	0.052	0.123	0.288	0.448	0.111
MDS post Rx (6)							
average	16.167	13.833	11.833	14.833	26.333	30.167	96.500
stdev	28.330	28.017	26.536	28.067	38.862	5.601	4.037
P value	0.474	0.394	0.427	0.457	0.230	0.113	0.754
PPARGC1B							
NC (21)							
average	4.333	7.000	4.714	3.158			
stdev	3.152	3.987	4.540	5.284			
MDS (20)							
average	6.950	10.850	9.400	12.706			
stdev	4.383	7.386	9.064	14.848			
P value	0.036	0.048	0.047	0.021			
MDS post Rx (6)							
average	4.167	5.500	5.500	5.167			
stdev	1.169	3.082	2.345	2.787			
P value	0.844	0.349	0.576	0.244			
WWP2							
NC (19)							
average	4.706	1.235	1.353	1.235	3.059	4.125	2.143
stdev	1.829	0.437	0.606	0.752	1.197	1.746	1.099
MDS (20)							
average	6.944	2.722	2.944	3.056	4.833	6.875	3.933
stdev	3.455	2.052	2.313	2.338	2.834	4.759	2.764
P value	0.023	0.007	0.011	0.005	0.023	0.043	0.032
MDS post Rx (6)							
average	3.667	2.000	1.667	1.667	3.000	4.667	3.333
stdev	0.577	1.000	1.155	0.577	1.000	1.528	1.155
P value	0.053	0.262	0.557	0.247	0.762	0.674	0.215



Supplementary Figure 1. (a) Induction of CD11b levels through 7 days of ATRA treatment. Light blue line indicates DMSO treated cells as a vehicle control; dark blue line indicates ATRA treated cells ($P = < 0.0001$ by interactive regression model comparing treatments over time) (b) Wright stained cytopsin slides of $1 \mu\text{M}$ ATRA treated HL60 cells on experimental days 0, 2, 4 and 7 ($40\times$). DMSO treated cells at day 7 are shown in the insert as a vehicle control.