Correlation of clinical response and response duration with miR-145 induction by lenalidomide in CD34+ cells from patients with del(5q) myelodysplastic syndrome

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Online Supplementary Appendix

Design and Methods

RNA expression after in vitro *exposure to lenalidomide*

Both cryopreserved human CB cells and patient marrow samples were separated based on CD34+ status by positive selection using magnetic beads (EasySep Human CD34 Positive Selection Kit, StemCell Technologies Inc., Vancouver, Canada). CD34+ and CD34- cells were cultured *in vitro* for 48 h with either lenalidomide (10 μM) or DMSO added to the culture media. The small and large RNA fractions were isolated using the mirVana PARIS kit (Ambion, Austin, TX, USA) and relative miRNA expression was determined by RT-qPCR. The purified large RNA preparation was used as a template to generate first strand cDNA using SuperScriptII (Invitrogen, Carlsbad, CA, USA). In a similar fashion, lineage-negative cells from mouse marrow were selected using magnetic beads (EasySep Mouse Hematopoietic Progenitor Cell Enrichment Kit, StemCell Technologies Inc., Vancouver, Canada). Mean fold-change values were compared using Student's t-test. For the correlation of fold change and clinical outcomes two-tailed Pearson's correlation was performed in all cases.

miRNA expression after transduction with decoy producing vector and response to lenalidomide re-exposure in vitro

To achieve knockdown of miR-143 and miR-145, we used miRNA decoys (target sequences for miR-143 and miR-145) as described previously.[1,2] Four tandem repeats of each miRNA seed recognition sequence were cloned into the 3'UTR of the GFP reporter gene in the lentiviral expression vector, LentiLox 3.7. CD34+ cells isolated from cryopreserved human cord blood cells by positive selection using magnetic beads were transduced with the decoy or control (empty) vector. GFP+ cells were flow-sorted. In examining the miRNA inhibition after transduction with the decoy-expressing vector, the mean values of 2 experiments are shown and are compared using Student's t-test. Clonogenic progenitor assays were then performed following treatment of transduced cells *in vitro* with lenalidomide (10 μM) or vehicle (DMSO) for 48 h as per manufacturer's instructions (Stem Cell Technologies, Vancouver, BC, Canada). Colony forming cells were scored after 14 days. Mean values of three experiments carried out in duplicate are presented and compared using Student's t-test.

Statistics for the *in vitro* experiments were performed using GraphPad Prism software version 5.00 and the correlation with clinical outcome was made using SPSS software version 20.

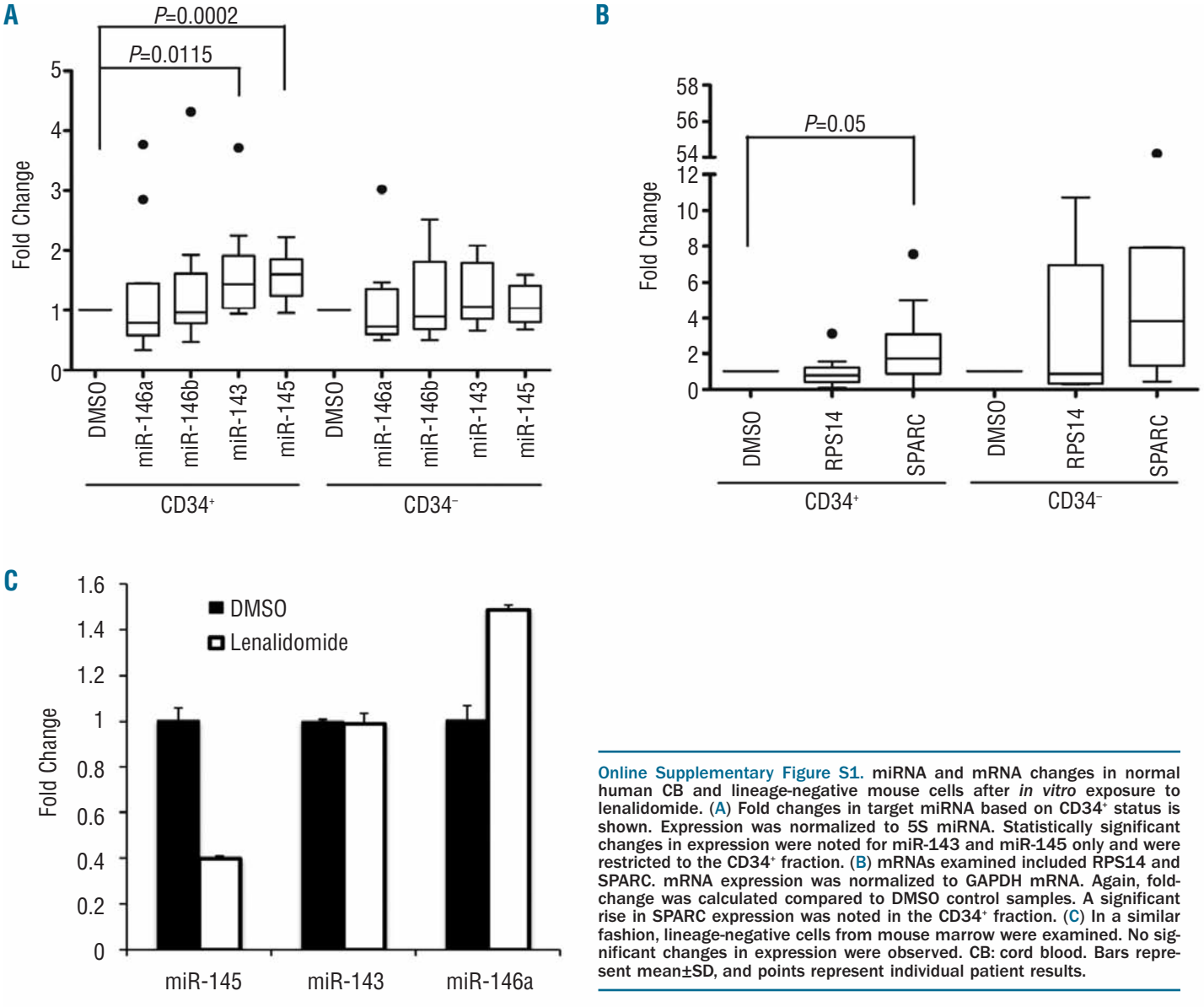
References

1. Starczynowski DT, Kuchenbauer F, Argiropoulos B, Sung S, Morin R, Muranyi A, et al. Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. Nat Med. 2010;16(1):49-58.
2. Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nature Methods. 2007; 4(9):721-6.

Online Supplementary Table S1. Patient's characteristics.

Del(5q) status	Patient	Age (years)	Sex	Cytogenetics	Blast (%)	IPSS score	IPSS	Previous treatment type	TI	Relapse	Response duration (months)
Del(5q)	1	71	F	del(5q), poor	1	1.5	INT-2	None	yes	no	31.3
Del(5q)	2	52	F	del(5q), int	1	1	INT-1	None	yes	yes	10.4
Del(5q)	3	44	F	del(5q), int	4	1	INT-1	ESA	yes	no	36.0
Del(5q)	4	78	F	46XX,del 5q(q13q31)	4	0.5	LR	ESA, Prinomostat, Thalidomide	yes	no	20.6
Del(5q)	5	75	M	46, xy, del (5q22-33)	4	0.5	LR	ESA, G-CSF, Thalidomide, ARA-C,	no		
Del(5q)	6	79	F	46,XX,del(5)(q13q33)[19]/46,XX[1]	6	0	LR	ESA	yes	yes	39.7
Del(5q)	7	61	F	46,XX,del(5)(q13q33)[4]/46,idem,del(1)(p34)[3]/46,XX[13]	2	0	LR	ESA	yes	yes	23.0
Del(5q)	8	83	M	del(5q)	<5	1	INT-1	ESA	yes	no	37.5
Del(5q)	9	74	F	del(5q)	3	1	LR	None	yes	yes	5.5
Del(5q)	10	56	F	del(5q)	2	1	INT-1	None	yes	no	17.1
Non-del(5q)	1	73	M	46 XY	1.8		LR	ESA, Prinomostat	yes		
Non-del(5q)	2	67	M	46 XY	1	0.5	INT-1	None	no		
Non-del(5q)	3	75	M	46 XY	0	0.5	INT-1	None	no		
Non-del(5q)	4	70	M	46 XY	9	1	INT-1	ESA, thalidomide and azacytidine	no		
Non-del(5q)	5	60	M	46, XY, inv (3)(q21, q26)(12/20)	9	0.5	INT-1	ESA	no		
Non-del(5q)	6	79	F	46 XX	0	0	LR	ESA	no		
Non-del(5q)	7	78	M	46 XY	6	1	INT-1	p38 MAP kinase inhibitor	no		
Non-del(5q)	8	58	M	46 XY	3	0	LR	ESA	no		
Non-del(5q)	9	67	M	46 XY	2	0.5	INT-1	ESA	no		
Non-del(5q)	10	82	M	46 XY	8	1	INT-1	ESA	no		
Non-del(5q)	11	81	M	45 X, -Y	2	0	LR	ESA and azacytidine	no		
Non-del(5q)	12	79	M	46 XY	1	0.5	INT-1	ESA	no		
Non-del(5q)	13	78	M	45 X, -Y	1	0	LR	ESA and G-CSF	no		
Non-del(5q)	14	67	M	46 XY	1	0	LR	p38 MAP kinase inhibitor	no		
Non-del(5q)	15	72	F	46 XX	2	0	LR	ESA and G-CSF	yes	yes	5.5
Non-del(5q)	16	76	M	46 XY	4	0	LR	ESA	yes	no	52.1
Non-del(5q)	17	68	M	46 XY	1	0.5	INT-1	ESA	no		
Non-del(5q)	18	75	M	45 X, -Y	<5	1	INT-1	ESA	yes	no	17.5

ESA: erythropoiesis stimulatng agent; G-CSF: granulocyte-colony stimulating factor; IPSS: International Prognostic Staging System; TI: transfusion independence.



Online Supplementary Figure S1. miRNA and mRNA changes in normal human CB and lineage-negative mouse cells after *in vitro* exposure to lenalidomide. (A) Fold changes in target miRNA based on CD34$^+$ status is shown. Expression was normalized to 5S miRNA. Statistically significant changes in expression were noted for miR-143 and miR-145 only and were restricted to the CD34$^+$ fraction. (B) mRNAs examined included RPS14 and SPARC. mRNA expression was normalized to GAPDH mRNA. Again, fold-change was calculated compared to DMSO control samples. A significant rise in SPARC expression was noted in the CD34$^+$ fraction. (C) In a similar fashion, lineage-negative cells from mouse marrow were examined. No significant changes in expression were observed. CB: cord blood. Bars represent mean±SD, and points represent individual patient results.