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Identification of a risk dependent microRNA expression signature in myelodysplastic syndromes

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Summary

The myelodysplastic syndromes (MDS) display both haematological and biological heterogeneity with variable leukaemia potential. MicroRNAs play an important role in tumour suppression and the regulation of self-renewal and differentiation of haematopoietic progenitors. Using a microarray platform, we evaluated microRNA expression from 44 patients with MDS and 17 normal controls. We identified a thirteen microRNA signature with statistically significant differential expression between normal and MDS specimens ($P < 0{\cdot}01$), including down-regulation of members of the leukaemia-associated *MIRLET7* family. A unique signature consisting of 10 microRNAs was closely associated with International Prognostic Scoring System (IPSS) risk category permitting discrimination between lower (Low/Intermediate-1) and higher risk (Intermediate-2/High) disease ($P < 0{\cdot}01$). Selective overexpression of *MIR181* family members was detected in higher risk MDS, indicating pathogenetic overlap with acute myeloid leukaemia. Survival analysis of an independent cohort of 22 IPSS lower risk MDS patients revealed a median survival of 3·5 years in patients with high expression of *MIR181* family compared to 9·3 years in patients with low *MIR181* expression ($P = 0{\cdot}002$). Our pilot study suggested that analysis of microRNA expression profile offers diagnostic utility, and provide pathogenetic and prognostic discrimination in MDS.

Keywords: microRNA, myelodysplastic syndromes, International Prognostic Scoring System, gene expression, haematopoiesis.

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of haematopoietic stem cell malignancies that share a high frequency of recurrent chromosomal aberrations and a complex pathogenesis (List *et al*, 2004). Pathogenic mechanisms underlying abnormalities of affected haematopoietic stem cells in MDS remain poorly characterized. Senescence-associated accumulation of genetic defects is believed to play a key role in the changes in regulation of apoptosis, differentiation and proliferation potential of affected progenitors (Bernasconi, 2008). MicroRNAs are short non-coding RNAs containing 19–25 nucleotides that impair translation or induce mRNA degradation of target mRNA (Fire *et al*, 1998; Bartel, 2004). Over 500 microRNAs have been identified in the human genome (Bentwich *et al*, 2005). Analysis of microRNA expression in haematological malignancies and solid tumours has identified expression patterns that are tumour type specific with prognostic relevance (Calin *et al*, 2002; Debernardi *et al*, 2007; Garzon & Croce, 2008; Garzon *et al*, 2008; Jongen-Lavrencic *et al*, 2008; Marcucci *et al*, 2008a). MicroRNA expression profiling has shown that myeloblasts from patients with acute myeloid leukaemia (AML) display an expression profile that is distinct from normal bone marrow progenitors and acute lymphoblastic leukaemia (ALL) (Mi *et al*, 2007). Marcucci and colleagues identified a microRNA signature with independent predictive power for event-free survival in cytogenetically normal AML, which included five members of the *MIR181* family that target genes involved in erythroid differentiation, homeobox genes and toll-like receptors (Marcucci *et al*, 2008a,b). To understand the role of microRNAs in MDS, we evaluated microRNA expression in bone marrow specimens from patients with MDS using a microarray platform and compared gene

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expression profiles to those obtained from normal donors. Gene expression was compared by International Prognostic Scoring System (IPSS) category (Greenberg *et al*, 1997) and test set risk signatures were confirmed in a validation set. We report microRNA class signatures with diagnostic discrimination that distinguish biological differences in MDS disease risk groups.

Methods

Patients and bone marrow specimens

Forty-four MDS and 17 normal control (NC) bone marrow mononuclear cells (BM-MNC) samples were obtained from Moffitt Cancer Center Tissue Procurement Repository. Written informed consent was obtained from all donors, and laboratory investigations were approved by the Institutional Review Board of the University of South Florida. MDS cases were characterized morphologically according to the World Health Organization (WHO) classification for myeloid malignancies (Swerdlow *et al*, 2008). Prognostic risk was assigned using the IPSS based on number of cytopenias in peripheral blood, percentage of bone marrow blasts and cytogenetics abnormalities (Greenberg *et al*, 1997). Clinical and demographic data are summarized in Table SI. BM-MNC were isolated from heparinized bone marrow aspirates on Ficoll-Hypaque gradient and cryopreserved in 20% fetal bovine serum with 10% dimethyl sulfoxide. After thawing cells were washed in phosphate-buffered saline before total RNA isolation.

RNA extraction and genome wide microRNA microarray profiling

Total RNA was isolated using QIAzol followed by RNeasy Mini kit (Qiagen, Valencia, CA, USA) according to manufacturer instructions. Genome-wide microRNA microarray profiling was performed using a microRNA microarray platform (OSU 3) consisting of 1100 microRNA probes in duplicates including 345 human and 249 mouse microRNA genes. microRNA isolated from haematopoietic cells was biotin-labelled and hybridized on microRNA chips as described elsewhere (Liu *et al*, 2004). Briefly, 5 μg of total RNA from each sample was reverse transcribed by using biotin end-labelled random octamers. Hybridization was carried out on the custom microRNA microarray chip (with probes length 40-b) spotted in quadruplicate with annotated active sites. The hybridized chips were washed and processed to detect biotin-containing transcripts by streptavidin-Alexa647 conjugate and scanned by using an Axon 4000B (Axon Instruments, Union City, CA, USA). Scanned images of chips were quantified by GENEPIX PRO 6·0 (Axon Instruments). Raw data were normalized using quantiles. MicroRNA was excluded when <20% of expression data had at least a 1·5-fold change in either direction from microRNA's median value.

Statistical analysis

Statistical comparisons were made using the BRB Array tools (Simon *et al*, 2007). Expression data were normalized using quantiles. Class comparison between groups of arrays used random variance *t* tests, which provides improved estimates of gene-specific variances without assuming that all microRNAs have the same variance. The criteria for inclusion of a microRNA was a *P*-value less than a specified threshold value (i.e. 0·01). For constructing predictors and classifying experiments into classes based on microRNA expression levels we used class prediction in BRB Array tools. Six methods of prediction were applied: compound covariate predictor, diagonal linear discriminant analysis, *k*-nearest neighbour (using $k = 1$ and 3), nearest centroid, and support vector machines. Class prediction determined cross-validated misclassification rate and performed a permutation test to determine if the cross-validated misclassification rate was lower than it would be expected by chance. The criterion for inclusion of a gene in the predictor was a *P*-value less than a specified threshold value (i.e. 0·01). The output contains the result of the permutation test on the cross-validated misclassification rate, and a listing of genes that comprise the predictor, with parametric *P*-values for each microRNA and the CV-support percentage (percentage of times when the microRNA was used in the predictor for a leave-one-out cross-validation procedure). Heatmaps were produced using centred correlation distance and average linkage with CLUSTER 3.0 (http://genome-www.stanford.edu) and visualized with JAVA TREEVIEW (Volinia *et al*, 2010). Survival curves were generated using Kaplan–Meier method and the log-rank test to test any difference of survival curves. Holm's method was used to adjust for the two tests.

Real time RT-PCR reverse transcription polymerase chain reaction (RT-PCR)

Single tube TaqMan MicroRNA Assays was performed to quantify mature microRNAs on Applied Biosystems Real-Time PCR instruments. All reagents, primers and probes were obtained from Applied Biosystems (Applied Biosystems, Foster City, CA, USA). RNU48 was used to normalize all RNA samples. Reverse Transcriptase Reactions and Real-Time PCR were performed according to the manufacturer's protocols except that we used half volumes, 7·5 μl for the RT reaction and 10 μl for the PCR reaction, respectively. RNA concentrations were determined with a NanoDrop (NanoDrop Technologies, Inc, Wilmington, DE, USA). One nanogram RNA per sample was used for the assays. All RT reactions, including no-template controls and RT minus controls, were run in a GeneAmp PCR 9700 Thermocycler (Applied Biosystems). Gene expression levels were quantified using the ABI PRISM 7900HT Sequence detection system (Applied Biosystems). Comparative real-time PCR was performed in triplicate, including no-template controls. Relative expression was calculated using the comparative C_t method (Chen *et al*,

2005). Wilcoxon test was used to compared values between groups, $P < 0{\cdot}05$ was considered significant.

MicroRNA locked nucleic acid in situ *hybridization (LNA-ISH) of formalin-fixed, paraffin-embedded bone marrow biopsy section*

In situ hybridization (ISH) was carried out on deparaffinized bone marrow tissues using a previously published protocol (Nuovo *et al*, 2009), which included digestion in pepsin (1·3 mg/ml) for 30 min. The sequences of the probes containing the six dispersed locked nucleic acid (LNA) modified bases with digoxigenin conjugated to the 5′ end were: *MIR181*-(5′) ACCCACCGACAGCAATGAATGTT (Exiqon, Inc, Woburn, MA, USA). The probe cocktail and tissue microRNA were co-denatured at 60°C for 5 min, followed by hybridization at 37°C overnight and a low stringency wash in 0·2× saline sodium citrate (SSC) and 2% bovine serum albumin at 4°C for 10 min. The probe-target complex was seen due to the action of alkaline phosphatase on the chromogen nitroblue tetrazolium and bromochloroindolyl phosphate (NBT/BCIP). Negative controls included the use of a scrambled probe. *MIR328* served as a positive control.

Results

We studied microRNA profiles in patients with MDS using microarray platform. Since a single microRNAs is able to regulate multiple genes simultaneously it is plausible that microRNAs are implicated in pathogenesis of such complex and heterogeneous disorders as MDS. We hypothesized that deregulation of microRNA expression is most probably initiated in a pluripotent MDS stem cell and subsequently affects more mature bone marrow cells represented in the fraction of bone marrow mononuclear cells. Although it would be optimal to study population of CD34+ cells this approach was not technically feasible because a microchip platform required 5 μg of total RNA for each sample analysis.

MicroRNA expression profile distinguishes MDS from normal haematopoiesis

MicroRNA expression was analysed in 44 MDS patient and 17 normal control specimens using a microRNA chip (OSU version 3). Overall, the MDS cohort included 66% patients with Low/Intermediate-1 risk IPSS categories, and 33% patients with Intermediate-2/High risk disease. WHO distribution included refractory anaemia (RA) ($n = 5$), refractory anaemia with ring sideroblasts (RARS) ($n = 8$), MDS with deletion 5q ($n = 6$), refractory cytopenia with multilineage dysplasia (RCMD) ($n = 7$), refractory anaemia with excess blasts-1 (RAEB-1) ($n = 10$) or RAEB-2 ($n = 8$), MDS unclassifiable (MDS-U) ($n = 4$), myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U) ($n = 2$). Overall, 290 microRNAs passed the filtering criterion; among which 42 were up-regulated and 34 down-regulated (Table SII). Fifteen microRNAs were selected among the 76 microRNAs with differential expression ($P < 0{\cdot}001$) according to class comparison from which an expression heat map was constructed (Fig 1). Using the class prediction we found that MDS and normal controls had distinct microRNA profiles. A signature consisting of thirteen microRNAs was sufficient to discriminate between these two cohorts (Table I). Included among these are two down-regulated microRNAs that have key roles in tumourigenesis through deregulation of gene targets such as *RAS*, *MYC*, *HMGA2* and *CASP3* (*MIRLET7* family) (Johnson *et al*, 2005; Park *et al*, 2007; Sampson *et al*, 2007; Tsang & Kwok, 2008) and stem/progenitor cell self-renewal and

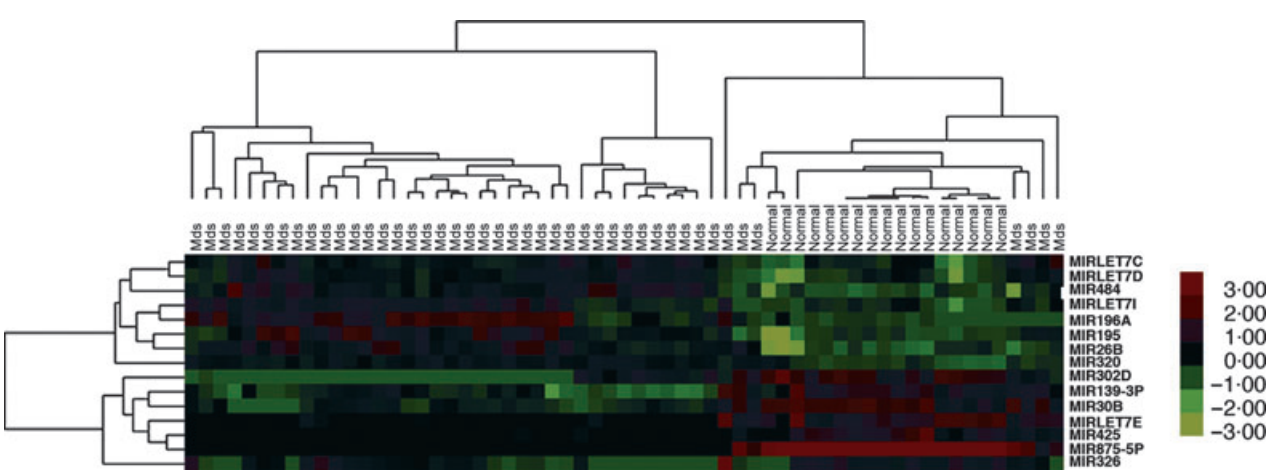

Fig 1. MicroRNAs can differentiate MDS and normal controls. Two-way hierarchical clustering of 44 MDS and 17 normal controls (NC) was performed after quality filtering. Hierarchical clustering showed clustering of the samples according to disease status. A heat map was generated using the expression ratios of 15 microRNAs selected out of total 73 that differed significantly ($P < 0{\cdot}001$). Red: up-regulated microRNAs, green: down-regulated microRNAs. Each column represents a MDS or NC sample, and each row represents a single microRNA. Patient samples are grouped by IPSS. The heatmap colour key gradient ranging from global minimum (green) −3 to global maximum (red) +3 is on the right side.

Table I. *MicroRNA* signature differentiating MDS *versus* Normal Controls (NC).

MicroRNA	Parametric *P* value	Fold change	Chromosomal location
MIR222	0·0020831	3·81	Xp11.3
MIR10A	0·0018187	3·37	17q21.32
MIR196A	6·66E-05	3·04	17q21.32
MIR320	<1e-07	2·12	8p21.3
MIR100	0·0298715	1·51	11q24.1
MIR124	0·0009909	0·60	8p23.1
MIR206	0·0093797	0·59	6p12.2
MIR146A	0·0356401	0·57	5q34
MIR150	0·0365018	0·54	19q13.33
MIR326	0·0001276	0·51	11q13.14
MIRLET7E	<1e-07	0·46	19q13.41
MIR197	0·000754	0·33	1p13.3
MIR875-5P	<1e-07	0·02	8q22.2

This 13 microRNA signature was predictive (misclassification error rate after 10-fold CV, <0·01). Red: up-regulated microRNAs. Green: down-regulated microRNAs.

maturation potential (*MIR146A*, *MIRLET7*) (Labbaye *et al*, 2008; Liu *et al*, 2009); in addition to increased expression of microRNAs that have repressive roles in erythroid (*MIR222*) and megakaryocyte (*MIR10A*) maturation (Felli *et al*, 2005; Garzon *et al*, 2006). Of particular interest, *MIR146A* is encoded on the long arm of chromosome 5 within the commonly deleted region associated with the 5q- syndrome (Starczynowski *et al*, 2010). Two of the up-regulated microRNAs are located within homeobox gene clusters that have important roles in haematopoietic development and oncogenesis. *MIR10A* and *MIR196A1* are located within the HOX B cluster on 17q21, whereas *MIR196A2* is within the HOX C cluster at 12q13 (Calin *et al*, 2004).

MicroRNA expression distinguishes higher from lower risk MDS

The microRNA expression patterns from 10 patients with higher risk (HR) MDS according IPSS score (Int-2/High) were compared to 10 patients with lower risk (LR; Low/Int-1) MDS. After normalization of data, differences in expression of 68 microRNAs were statistically significant (38 up-regulated and 30 down-regulated microRNAs, $P < 0·05$) (Table SIII). Thirty of these microRNAs were selected for heat map construction (Fig 2). Class prediction analysis identified a unique microRNA signature consisting of nine up-regulated and one down-regulated microRNAs that discriminated the two prognostic groups (Table II). Four members of the *MIR181* family were up-regulated in HR MDS specimens.

Results from the microchip platform were validated on an independent set of 18 samples (Table SIV) using quantitative RT-PCR. Expression of seven randomly selected microRNAs was evaluated (Fig 3) and confirmed the findings from the microarray chip. Next, we examined expression of *MIR181B* in bone marrow biopsies using *in* situ hybridization with locked nucleic acid (LNA)-modified probes in six bone marrow biopsies from MDS patients and two controls. *MIR181B* was strongly expressed in the cytoplasm of HR MDS cells (Fig 4), whereas specimens from LR MDS displayed reduced staining intensity. Although a cohort of our MDS patients was heterogeneous and included the six most common MDS subtypes according to WHO classification our results suggested that there is an overlap in molecular pathogenesis between these subtypes.

Expression of MIR181 family correlates with survival of patients with LR MDS

We also evaluated overall survival in an independent cohort of 22 MDS patients with IPSS lower risk. Results of this analysis revealed a significant difference in survival of patients with high expression of *MIR181* family, with a median survival of 3·5 years compared to 9·3 years in patients with low *MIR181* expression ($P = 0·002$) (Fig 5). Interestingly, two of three patients in the data set that developed AML had up-regulation of the *MIR181* family, whereas one did not.

Discussion

We have identified and validated two microRNA signatures that discriminate MDS from normal control subjects and patients with HR from LR MDS, respectively. A vast majority of microRNAs included in both signatures were previously found to be implicated in regulation of haematopoiesis, apoptosis and angiogenesis.

The *MIR181* family plays an important role in the negative regulation of haematopoiesis including the proliferation and the differentiation of stem/progenitor cells and megakaryocytic lineage development (Georgantas *et al*, 2007). A putative role for *MIR181* in the pathobiology of myeloid malignancies is supported by a recent report by investigators in the Cancer and Leukemia Group B (CALGB) showing that expression of five members of *MIR181* family among a 12 microRNA signature was inversely associated with progression-free survival in AML patients with normal cytogenetics (Marcucci *et al*, 2008b).

Several microRNAs involved in stage-specific control of erythropoiesis (Choong *et al*, 2007; Georgantas *et al*, 2007) were deregulated in MDS. Expression of *MIR221* and *MIR222* normally decline with erythroid maturation to de-repress *KIT* expression and facilitate erythroblast expansion (Felli *et al*, 2005). Overexpression of these microRNAs, which distinguished MDS patients from controls in our study, can suppress erythroid growth and may be operative in disease-associated ineffective erythropoiesis. (Table II). The latter may be compounded by down-regulation of members of the *MIR144/451* cluster in HR MDS, which are key transcriptional targets of the erythroid transcription factor GATA-1, which are normally up-regulated with late stages of differentiation (Dore

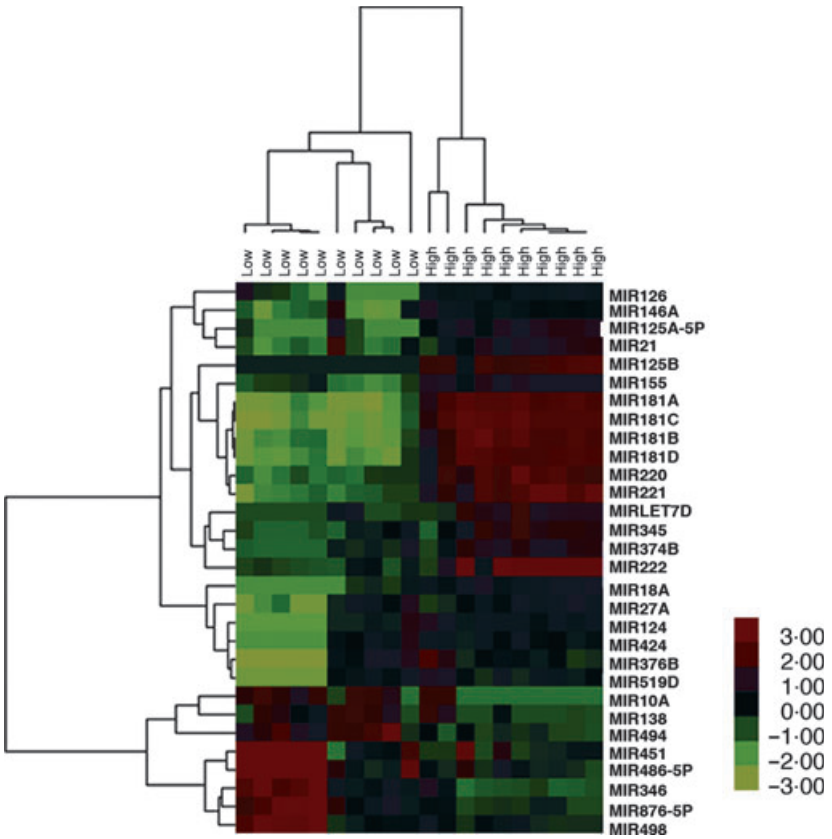

Fig 2. MicroRNAs can differentiate MDS according to prognosis. Hierarchical clustering of 10 high risk and 10 low risk MDS samples revealed a clustering according to prognostic group. A heat map was generated using the expression ratios of 30 microRNAs selected out of total 68 that differed significantly ($P < 0{\cdot}05$). Red: up-regulated microRNAs, green: down-regulated microRNAs. Each column represents a patient sample, and each row represents a single microRNA. Patient samples are grouped by IPSS. The heatmap colour key gradient ranging from global minimum (green) −3 to global maximum (red) +3 is on the right side.

Table II. *MicroRNA* signature differentiating HR *versus* LR MDS.

MicroRNA	Parametric *P* value	Fold change	Chromosomal location
MIR181C	<1e-07	22·72	19p13.13
MIR181A	<1e-07	19·95	19q33.3
MIR181B	2E-07	14·25	1q32.1
MIR181D	<1e-07	13·88	19p13.13
MIR221	4E-07	12·07	Xp11.3
MIR376B	0·024925	4·99	14q32.31
MIR125B	9E-07	4·27	11q24.1
MIR155	0·000131	3·62	21q21.3
MIR130A	0·000675	2·80	11q12.1
MIR486-5P	0·005686	0·14	8p11.21

The signature was predictive (misclassification error rate after 10-fold CV, <0·01). Red: up-regulated microRNAs, green: down-regulated microRNAs.

et al, 2008). *MIR155*, a translational repressor of several myeloid transcription factors including PU.1, C/EPBβ and CSF1R (Georgantas *et al*, 2007; O'Connell *et al*, 2008), was significantly up-regulated in HR MDS. Mice transplanted with *MIR155* transfected stem cells developed a myeloproliferative disorder with abnormal granulocyte morphology analogous to MDS, suggesting a role in the higher risk MDS phenotype (O'Connell *et al*, 2008). Recent investigations have shown that the proliferative effects of *MIR155* are mediated in part through down-regulation of the phosphatase and tensin homologue (PTEN) and consequent up-regulation of the programmed cell death protein 4 (PDCD4), Src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP1) and Akt phosphorylation (Yamanaka *et al*, 2009). Although not included in the diagnostic signatures, we found down-regulation of two *MIR29* family members in MDS specimens; *MIR29B* in HR MDS and *MIR29A* in MDS compared to normal controls. Both microRNAs are located on chromosome 7q32, a region commonly deleted in MDS and therapy-related AML (Le Beau *et al*, 1986). These microRNAs target *MCL1*, a member of the BCL2 anti-apoptotic gene family that is overexpressed in AML and HR MDS (Mott *et al*, 2007; Economopoulou *et al*, 2010), as well as the DNA methytranferases DNMT-3A and -3B, and indirectly via Sp1, DNMT-1 (Fabbri *et al*, 2007; Garzon *et al*, 2009). Loss of *MIR29A/B*

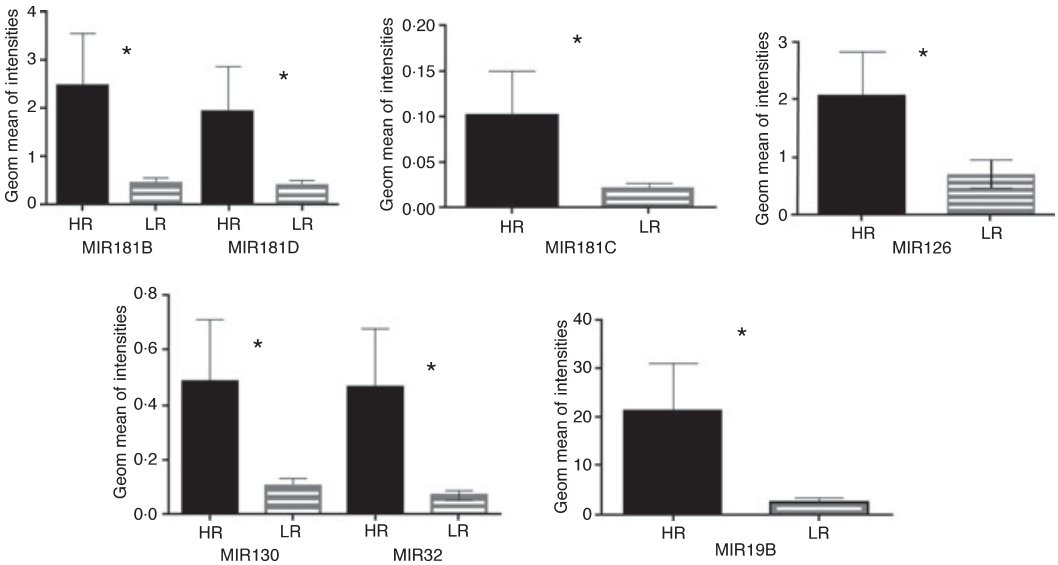

Fig 3. Validation of microRNA expression using independent sample set by Real -Time RT-PCR. Samples from 18 independent MDS patients (HR = 6, LR = 12) were analysed. Seven differentially expressed microRNAs were randomly selected for Real-Time RT-PCR. Significant correlation was demonstrated for all tested microRNA between microRNA microchip platform and qRT-PCR.

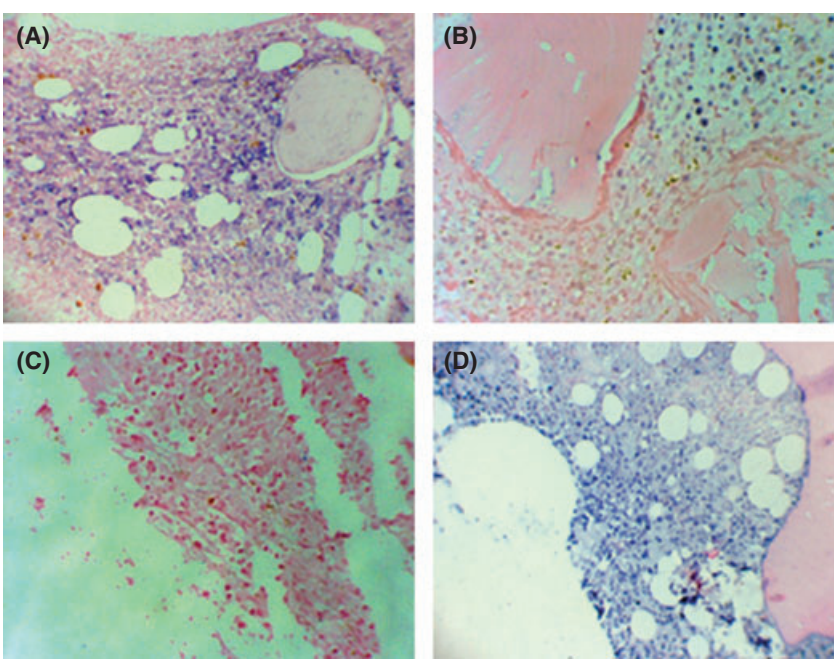

Fig 4. MicroRNA181B locked nucleic acid *in situ* hybridization (LNA-ISH) of formalin-fixed, paraffin-embedded bone marrow biopsy sections. We examined six patients with MDS and two controls. *MIR181B* is present in the cytoplasm of both (A) HR (+++) and (B) LR MDS (++) cells arranged in clusters on the background of normal (*MIR181B* negative) haematopoietic cells. (C) Negative control – scrambled microRNA probe. (D) Positive control –*MIR328* (A 200×, B 400×, C 400×, D 200×).

results in over-expression of *MCL1* and *DNMT*s, with corresponding aberrant genomic methylation, suggesting that down-regulation of the *MIR29* family may be a key pathogenetic feature of MDS, and as recently reported, progression of the disease to AML (Jiang *et al*, 2009). Megakaryocytic differentiation of common myeloid progenitors is tightly regulated by the coordinated down-regulation of *MIR10A, MIR126, MIR130A* and *MIR155*, and up-regulation of *MIR150* (Liu *et al*, 2004; Garzon *et al*, 2006; Yendamuri & Calin, 2009). Induction of *MIR150*, which suppresses expression of c-myb, is

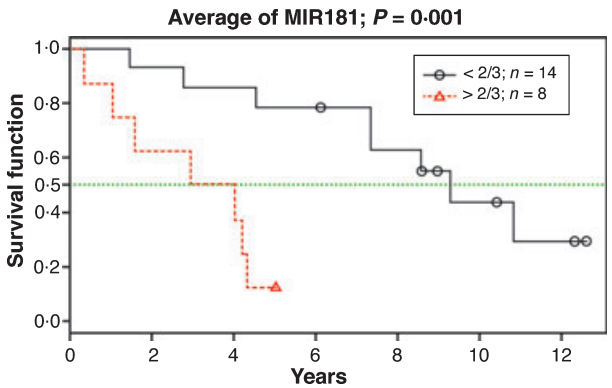

Fig 5. Kaplan–Meier estimate of overall survival in 22 IPSS lower-risk MDS patients by *MIR181* family expression. Four microRNAs (*MIR181A, MIR181B, MIR181C, MIR181D*) from the 10 microRNA prognostic signature were selected due to the wide variance in gene expression (>10-fold). Since family member expression levels were highly correlated ($r > 0{\cdot}8$), a composite score was generated by averaging four microRNAs separate standardized scores. Anticipating that about one-third of the patients should be considered as having 'high risk' due to high microRNA expression, we divided the patients into two groups, comparing the 14 patients with lower scores, to the eight patients with higher scores. Results showed a significant difference in survival from date of diagnosis to date of death or last visit.

critical for megakaryocytic lineage commitment (Barroga *et al*, 2008; Lu *et al*, 2008). Our findings of down-regulation of *MIR150* accompanied by up-regulation of *MIR10A* in MDS patient specimens compared to normal controls suggests a possible role in dysmegakaryopoiesis and impaired thrombopoiesis in MDS. Interestingly, *MIR155, MIR126* and *MIR130* were overexpressed in HR-MDS, which may further suppress megakaryopoiesis and account for the higher frequency of thrombocytopenia that occurs with disease progression (Garzon *et al*, 2006). Further investigation is warranted to determine if these microRNA changes are associated with dysplastic megakaryocytopoiesis and severity of thrombocytopenia in MDS patients.

The elaboration of angiogenic molecules has been implicated in autocrine cytokine networks supporting myeloid progenitor self-renewal and in bone marrow microvessel density in HR MDS (Bellamy *et al*, 2001). Several up-regulated microRNAs in HR MDS, including *MIR27B, 155, 21, 126* and *130A* were previously implicated in regulation of different stages of angiogenesis (Urbich *et al*, 2008). *MIR130A* targets a site in the 3′-UTR of the anti-angiogenic homeobox gene *HOXA5* to promote angiogenesis (Chen & Gorski, 2008). In a zebrafish model *MIR126* was shown to control vascular integrity and micro-vessel formation by repressing negative regulators of the vascular endothelial growth factor (VEGF) signalling receptor pathway (Fish *et al*, 2008). Our data suggest that changes in microRNA expression may promote angiogenesis and lead to MDS disease progression. Although it is difficult to draw firm conclusions from our prognostic and diagnostic signatures in terms of a mechanism of action, our pilot study showed that putative microRNA targets obtained from published literature or from *in silico* prediction models are implicated in the regulation of multiple important pathogenic pathways in haematopoiesis and MDS. However, further studies are needed to determine whether selective targeting of these microRNAs might alter disease phenotype and outcome.

Conclusions

Our data reveal that changes in microRNA expression may have a fundamental role in the pathogenesis and phenotype of MDS. The vast majority of alterations in microRNA expression affected microRNAs implicated in haematopoiesis and tumourigenesis. MicroRNA expression profiling was able to discriminate between normal and MDS haematopoiesis, suggesting that molecular characterization may offer a potentially more rigorous diagnostic alternative to the reliance on morphological recognition with direct biological implications. Moreover, the identification of microRNA signatures that distinguish IPSS LR from HR MDS indicates that microRNA expression profile also offers prognostic utility that might, in the future, provide insight into appropriate therapeutic selection. Additionally, *MIR181*expression may offer a molecular tool to refine discrimination of disease behaviour. Further investigations done on a large population of MDS patients are necessary to discern the possible relationship between microRNA profile and haematological phenotype, therapeutic response and overall survival.

Acknowledgements

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Supporting information

Additional Supporting information may be found in the online version of this article:

Table SI. Patient Samples/Clinical Data/Microarray Platform.

Table SII. MiRNA Expression Profiling MDS ***versus*** NC.

Table SIII. MiRNAs Expression Profiling MDS HR ***versus*** LR.

Table SIV. Patient Samples/Clinical Data/Real-Time RT-PCR.

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