Epigenetic inactivation of the *hsa-miR-203*
in haematological malignancies

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Abstract

miR-203 is a tumour suppressor microRNA (miRNA). We studied the methylation of *hsa-miR-203* in 150 samples including acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL) and non-Hodgkin's lymphoma (NHL) by methylation-specific PCR, and miRNA expression by stem-loop RT-qPCR. *hsa-miR-203* promoter was unmethylated in normal controls but homozygously methylated in two AML and four lymphoma cell lines, in which 5-Aza-2′-deoxycytidine treatment led to promoter demethylation and *miR-203* re-expression. Restoration of *miR-203* expression in lymphoma cells inhibited cellular proliferation and increased cell death, suggesting an inherent tumour suppressor activity. In primary samples, *hsa-miR-203* methylation was absent in CML but detected in 5.0% ALL, 10.0% AML, 42.0% CLL and 38.8% of NHL (including six [60.0%] natural killer-cell, nine [40.9%] B-cell and four [23.5%] T cell NHL). Moreover, *hsa-miR-203* methylation was associated with hypermethylation of *hsa-miR-34a, -124a* and *-196b* in NHL but not CLL. In CLL, *hsa-miR-203* methylation was associated with a higher presenting Hb level (*P* = 0.033). The projected 10 year overall survival of the CLL patients was 58.2%, which was impacted by Rai stage and high-risk karyotypes but not *hsa-miR-203* methylation. *hsa-miR-203* was more frequently methylated in lymphoid than myeloid malignancies (*P* = 0.002). In conclusion, *miR-203*, a tumour suppressor gene, was hypermethylated in a tumour-specific manner with gene silencing. *hsa-miR-203* was more frequently hypermethylated in lymphoid than myeloid malignancies. In NHL, *hsa-miR-203* methylation was associated with concomitant methylation of other tumour suppressor miRNAs. The frequent *hsa-miR-203* methylation in lymphoid malignancies suggested a pathogenetic role of *hsa-miR-203* methylation.

Keywords: microRNA ● tumour suppressor ● hypermethylation ● leukaemia ● lymphoma

Introduction

DNA methylation involves the addition of a methyl group to the number 5 carbon of the cytosine ring in the CpG dinucleotide, by catalysing the conversion of cytosine into methylcytosine through DNA methyltransferase [1, 2]. Cancer cells are characterized by global DNA hypomethylation but gene-specific hypermethylation of promoter-associated CpG islands of tumour suppressor genes (TSGs), resulting in transcriptional repression, and hence serve as an alternative mechanism of gene inactivation. Based on a pathway-specific approach, multiple TSGs across pathways including cell cycle regulation, Janus kinase/signal transducers and activators of transcription (JAK/STAT) signalling, WNT signalling and death-associated protein (DAP) kinase-associated intrinsic tumour suppression have been shown to be inactivated by gene hypermethylation in leukaemia, lymphoma and multiple myeloma [3–9].

MicroRNA (miRNA) is a single-stranded, non-coding RNA molecule of 22–25 nucleotides, which leads to down-regulation of target protein expression [10]. miRNAs are involved in carcinogenesis. miRNAs can be either oncogenic (oncomir) when TSGs are targeted, or tumour suppressive (tumour suppressor miRNAs)

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when oncogenes are targeted [10, 11]. Little is known about the role of hypermethylation of tumour suppressor miRNAs in haematological cancers.

Recently, hypermethylation of *hsa-miR-203* has been reported in chronic myeloid leukaemia (CML) and hepatocellular carcinoma, conferring proliferative advantage in tumour cells [12, 13]. Specifically, restoration of *miR-203* expression targets and downregulates the oncogenic breakpoint cluster region-abelson (BCR-ABL) fusion protein, thereby inhibits cellular proliferation in CML, demonstrating the tumour suppressor role of *miR-203* [12].

In this study, we aimed to study the role of *hsa-miR-203* methylation in a wide range of haematological malignancies including acute myeloid leukaemia (AML), CML, acute lymphoblastic leukaemia (ALL), chronic lymphocytic leukaemia (CLL) and non-Hodgkin's lymphoma (NHL).

Materials and methods

Patient samples

Diagnostic bone marrow or tissue samples were obtained in 20 ALL, 20 AML, 11 Ph$^+$ (expressing BCR-ABL) CML in chronic phase, 50 CLL and 49 NHL patients. Diagnosis of leukaemia and lymphoma were made according to the French-American-British Classification and WHO Classification of Tumours, respectively [14–17]. Of the 20 ALL patients, there were 11 males and 9 females patients with a median age of 35 years (range: 13–62). There were six common ALL, one early B precursor, ten precursor B ALL and three pre-T ALL. Of the 20 AML patients, there were 9 males and 11 females with a median age of 41.5 years (range: 20–72). The AML cases comprised three M1, fourteen M2, two M4 and one M5 FAB subtype. Of the 50 CLL patients, there were 31 (62%) patients with limited Rai stage (<stage II) and 19 (38%) with advanced Rai stage (≥ stage II) disease with a median age of 64 years (range: 37–91). Thirty-seven (74%) were male. The median presenting lymphocyte count was 17×10^9/l (range: $10–236 \times 10^9$/l). Of the 37 patients with cytogenetic data, 9 (18%) carried high-risk cytogenetic alterations [del(17p), $N = 2$; trisomy 12, $N = 7$] and 28 (56%) carried low/standard-risk cytogenetic alterations (del[13], $N = 5$; normal karyotype, $N = 18$; other karyotypic changes, $N = 5$). Projected 10 year overall survival (OS) of CLL patient was 58.2% for the whole group, and 65.9% in those with limited, and 47.5% in those with advanced Rai stage ($P = 0.04$). Projected 10 year OS of CLL patient with low/standard-risk and high-risk karyotypes were 80.6% and 13.9% ($P = 0.001$). Of the 49 patients with NHL, the median age was 59.6 years (range: 17–86 years). There were 17 (34.7%) patients with peripheral T cell lymphoma (two anaplastic large cell, 4 angio-immunoblastic T cell, 11 peripheral T cell, not otherwise specified), 10 (20.4%) with natural killer (NK)/T cell lymphoma, nasal type, 22 (44.9%) patients with B-cell lymphoma (9 follicular: grade 1 to 2, 9 nodal marginal zone, and two each for mantle cell and diffuse large B-cell lymphoma). The study has been approved by Institutional Review Board of Queen Mary Hospital with informed consent.

Cell lines and culture

CLL cell lines EHEB, MEC-1 and lymphoma cell lines SU-DHL-6, SU-DHL-16, GRANTA-519, JEKO-1 and MINO were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). AML cell line SKNO-1 was purchased from Japanese Collection of Research Bioresources (Osaka, Japan). AML cell line HL-60 was kindly provided by Dr. R. Pang, Department of Medicine, HKU. CML cell lines K-562 and MEG-01 were kind gift from Dr. M. Yang, Department of Paediatrics, HKU. Cell lines were maintained in 90% RPMI 1640 + 10% FBS (HL-60, K-562, MEG-01, EHEB), 90% RPMI 1640 + 10% FBS + 10 ng/ml GM-CFS (SKNO-1), 90% IMDM + 10% FBS (MEC-1) and 85% RPMI 1640 + 15% FBS (SU-DHL-6, SU-DHL-16, GRANTA-519, JEKO-1 and MINO). Culture media were supplemented with 50 U/ml penicillin and 50 μg/ml streptomycin (Invitrogen, Carlsbad, CA, USA), and maintained in a humidified atmosphere of 5% CO_2 at 37°C.

Methylation-specific polymerase chain reaction (MSP)

DNA was extracted from bone marrow samples of ALL, AML, CML and CLL at diagnosis, diagnostic tissues (either lymph node or nasal biopsy in nasal NK-cell lymphoma) in patients with NHL, and cell lines by standard method. MSP for aberrant gene promoter methylation was performed as previously described [18]. Treatment of DNA with bisulphite for conversion of unmethylated cytosine to uracil (but unaffecting methylated cytosine) was performed with a commercially available kit (EpiTect Bisulphite Kit, QIAGEN, Hilden, Germany). Methylated MSP (M-MSP) primers were forward: 5′-GAG TAT TTT CGG TTT AGA CGA GAC-3′; reverse: 5′-CCT TTT ATA CGA CGC AAC CG-3′. Unmethylated MSP (U-MSP) primers were forward: 5′-TTT GAG TAT TTT TGG TTT AGA TGA GAT-3′; reverse: 5′-AAC ACC TTT TAT ACA ACA CAA CCA-3′. DNA from normal bone marrow donors ($N = 8$) was used as negative control, whereas enzymatically methylated control DNA (CpGenome Universal Methylated DNA, Chemicon, Temecula, CA, USA) was used as positive control in all the experiments. MSP was performed in a thermal cycler (9700, Applied Biosystems, Foster City, CA, USA) with the following cycling conditions: 95°C for 5 min., 35 cycles of 95°C for 30 sec., 58°C for 30 sec., 72°C for 30 sec. and a final extension of 10 min. at 72°C. The MSP mixture contained 50 ng of bisulphite-treated DNA, 0.2 mM deoxyribonucleotide triphosphates (dNTPs), 1.5 mM $MgCl_2$, 10 pmol of each primer, 1× PCR buffer and 2.5 units of AmpliTaq Gold DNA Polymerase (Applied Biosystems) in a final volume of 25 μl. Ten microlitres of PCR products were loaded onto 6% non-denaturing polyacrylamide gels, electrophoresed and visualized under ultraviolet light after staining with ethidium bromide.

5-Aza-2′-deoxycytidine (5-AzadC) treatment

For treatment with 5-AzadC (Sigma-Aldrich, St. Louis, MO, USA), cells were seeded in six-well plates at a density of 1×10^6 cells/ml, and cultured with 1 μM of 5-AzadC for 3 days. Cells on day 0 and day 3 of 5-AzadC treatment were harvested.

RNA isolation and stem-loop reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated using *mir*Vana^{TM} miRNA Isolation Kit (Ambion, Austin, TX, USA), according to the manufacturer's instructions. RT was

performed with Taqman® MicroRNA RT Kit and Taqman® MicroRNA Assay Kit (Applied Biosystems), according to the manufacturer's instructions. Total RNA was reverse transcribed in 1 mM dNTPs, 50 U MultiScribe™ Reverse Transcriptase, $1\times$ RT Buffer, 3.8 U RNase Inhibitor and $1\times$ stem-loop RT primer at following thermal cycling condition: 16°C for 30 min., 42°C for 30 min. and 85°C for 5 min. RT-qPCR of *miR-203* was performed in triplicate using 1.33 μl of 1:15 diluted RT product in $1\times$ Taqman® Universal PCR Master Mix and $1\times$ Taqman® Assay at 95°C for 10 min., followed by 40 cycles of 95°C for 15 sec. and 60°C for 1 min. RNU48 was used as reference for data analysis using the $2^{-\Delta\Delta Ct}$ method [19].

Mature *miR-203* overexpression

Mature *miR-203* RNA oligonucleotide mimic (100 nM) (Ambion) was transfected into 1×10^6 cells using X-tremeGENE siRNA Transfection Reagent (Roche, Basel, Switzerland), according to manufacturers' instructions. Control experiments were performed with non-targeting precursor mimic, negative control #1.

MTT assay

Cell proliferation was determined by colorimetric quantification of purple formazan formed from the reduction of yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) by proliferative cells. In brief, cells were seeded in a 96-well microtitre plate at 2.5×10^4 /well in 100 μl of medium. At the assay time-point, each well was added with 10 μl of 5 mg/ml MTT reagent and incubated for a further 4 hrs. Each well was added with 100 μl of dimethyl sulfoxide (DMSO). The absorbance reading at 550 nm with reference to 650 nm was recorded.

Trypan blue exclusion assay

Dead cells were measured by trypan blue exclusion method under microscope. Dead cells (%) = (total number of dead cells per microscopic field/total number of cells per microscopic field) $\times$ 100. Five random microscopic fields were counted in each sample.

Statistical analysis

The frequency of *hsa-miR-203* methylation in different types of haematological cancers was computed by chi-square or Fisher's exact test. In CLL, correlation between *hsa-miR-203* methylation status with continuous (mean age, mean diagnostic haemoglobin, lymphocyte and platelet counts) and categorical variables (gender, Rai staging, high-risk cytogenetic aberrations and methylation of *hsa-miR-34a*, *-124a* and *-196b*) were studied by Student's t-test and chi-square test (or Fisher's exact test), respectively. OS is measured from the date of diagnosis to the date of last follow-up or death. In CLL, OS of patients with limited Rai stage (stages 0, I and II) were compared to those with advanced stage (stages III and IV). Survival is plotted by the Kaplan–Meier method and compared by the log-rank test. All *P*-values were two sided. In NHL, correlation between *hsa-miR-203* methylation with continuous (mean age) and categorical variables (gender, lineage [B, T or NK/T], nodal/extranodal presentation and methylation of *hsa-miR-34a*, *-124a* and *-196b*) were studied by Student's t-test and chi-square test (or Fisher's exact test), respectively.

Results

MSP

Controls

None of the eight normal control marrows showed aberrant methylation of *hsa-miR-203* by MSP (Fig. 1A). The positive and negative controls showed the expected MSP results (normal DNA: U-MSP$^+$/M-MSP$^-$; methylated DNA: U-MSP$^-$/M-MSP$^+$).

Leukaemia and lymphoma cell lines

The profile of methylation of *hsa-miR-203* of leukaemia and lymphoma cell lines was shown in Fig. 1B. SU-DHL-6, SU-DHL-16, JEKO-1, MINO, HL-60 and SKNO-1 were homozygously methylated for *hsa-miR-203*.

Primary samples at diagnosis

Direct sequencing of the M-MSP products from the methylated primary samples showed the expected nucleotide changes after bisulphite treatment, therefore confirming complete bisulphite conversion and specificity of MSP (Fig. 2A). *hsa-miR-203* hypermethylation was not detected in any of the CML. On the other hand, *hsa-miR-203* methylation was preferentially found in NHL patients with methylation occurring in 1 (5%) ALL, 2 (10%) AML, 21 (42%) CLL and 19 (38.8%) NHL samples ($P < 0.0001$) (Fig. 2B). *hsa-miR-203* was more frequently hypermethylated in lymphoid than myeloid malignancies ($P = 0.002$). In CLL patients, the mean survival for the whole group was 86.97 months (89.28 months and 76.71 months for those with or without *hsa-miR-203* methylation). Moreover, *hsa-miR-203* methylation was associated with a higher mean Hb level than the unmethylated patients (12.2 g/dl *versus* 10.4 g/dl, $P = 0.03$) but not diagnostic lymphocyte count ($P = 0.98$) and platelet count ($P = 0.55$). Moreover, there was no correlation between *hsa-miR-203* methylation and age ($P = 0.38$), gender ($P = 0.99$), advanced Rai stage ($\geq$stage 2) ($P = 0.14$), high-risk karyotypic aberrations ($P = 0.99$) or death ($P = 0.76$). The projected OS in CLL patients with and without *hsa-miR-203* methylation were 69% and 62% ($P = 0.76$). Amongst lymphoma samples, *hsa-miR-203* was methylated in 9 (40.9%) B-cell NHL, 4 (23.5%) T cell and 6 (60%) NK/T cell NHL ($P = 0.165$). However, *hsa-miR-203* methylation did not correlate with age ($P = 0.74$), gender ($P = 0.24$), nodal/extranodal presentation ($P = 0.383$) or Ann Arbor stage ($P = 0.118$) of the lymphoma patients.

Associations of *hsa-miR-203* methylation with *hsa-miR-34a*, *-124a* and *-196b* methylation

In the 50 cases of CLL, there were 2 (4%) with *hsa-miR-34a*, 7 (14%) with *hsa-miR-124a* and 3 (6%) with *hsa-miR-196b* methylation. There was no association of *hsa-miR-203* methylation with
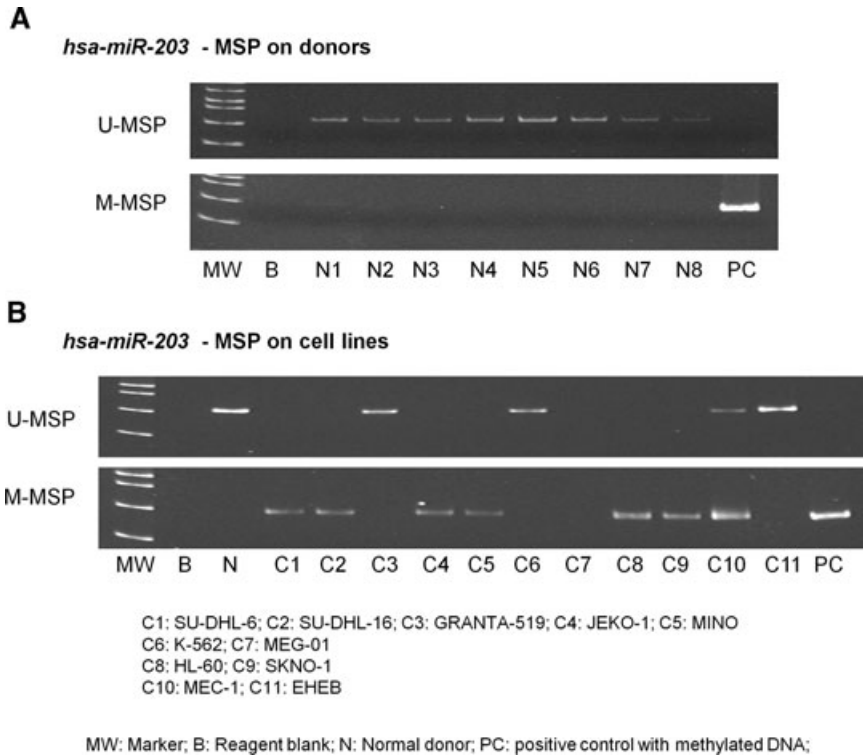
A

hsa-miR-203 - MSP on donors

B

hsa-miR-203 - MSP on cell lines

C1: SU-DHL-6; C2: SU-DHL-16; C3: GRANTA-519; C4: JEKO-1; C5: MINO
C6: K-562; C7: MEG-01
C8: HL-60; C9: SKNO-1
C10: MEC-1; C11: EHEB

MW: Marker; B: Reagent blank; N: Normal donor; PC: positive control with methylated DNA;

Fig. 1 Methylation of *hsa-miR-203*. (**A**) U-MSP showed that the methylated control [M] was totally methylated, and all eight normal controls (N1–N8) were unmethylated. In the M-MSP, the methylated control was positive (methylated) but all normal controls were negative (unmethylated). (**B**) For the cell lines, SU-DHL-6, SU-DHL-16, JEKO-1, MINO, HL-60 and SKNO-1 were completely methylated of *hsa-miR-203*.

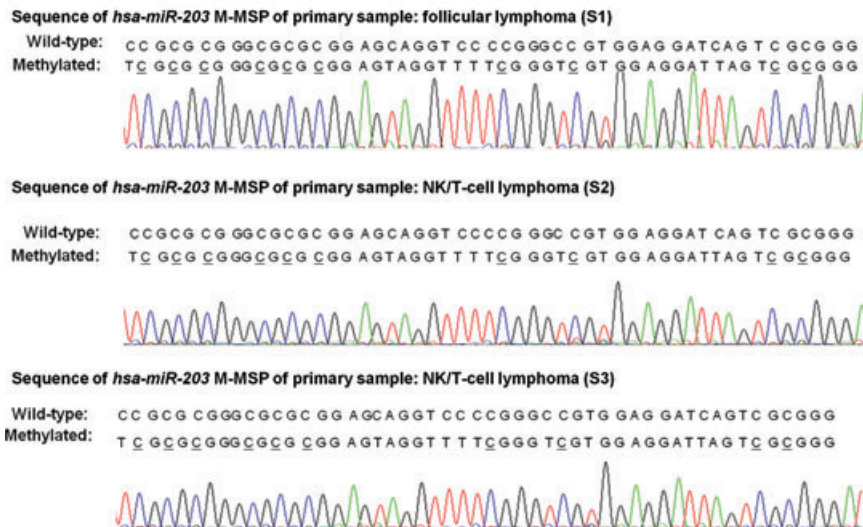## A   Primary samples - Direct sequencing of *hsa-miR-203* M-MSP products

Fig. 2 Methylation of *hsa-miR-203* in primary samples. (**A**) Sequence analysis of the M-MSP product from bisulphite-treated DNA showed that the cytosine [C] residues of CpG dinucleotides were methylated and remained unchanged, whereas all the other C residues were unmethylated and were converted to thymidine [T], indicating complete bisulphite conversion and specificity of MSP. (**B**) M-/U-MSP analysis of *hsa-miR-203* methylation in primary samples of haematological malignancies. (**C**) Correlation of *hsa-miR-203*, *-34a*, *-124a* and *-196b* methylation in samples as shown by M-MSP analysis from four representative NHL patients (B: reagent blank; S: primary sample; N: normal control; PC: positive control with methylated DNA).

hsa-miR-34a ($P = 0.171$), *-124a* ($P = 0.99$), *-196b* methylation ($P = 0.565$). In patients with NHL, 31 (63.3%) had concomitant study of the methylation status of *hsa-miR-34a*, *-124a* and *-196b* in addition to *hsa-miR-203*. There were 5 (16.13%) with *hsa-miR-34a*, 10 (32.26%) with *hsa-miR-124a* and 12 (38.7%) with *hsa-miR-196b* methylation. *hsa-miR-203* methylation was associated with *hsa-miR-34a* ($P = 0.027$), *hsa-miR-124a* ($P = 0.004$) and *hsa-miR-196b* methylation ($P = 0.021$) (Fig. 2C).
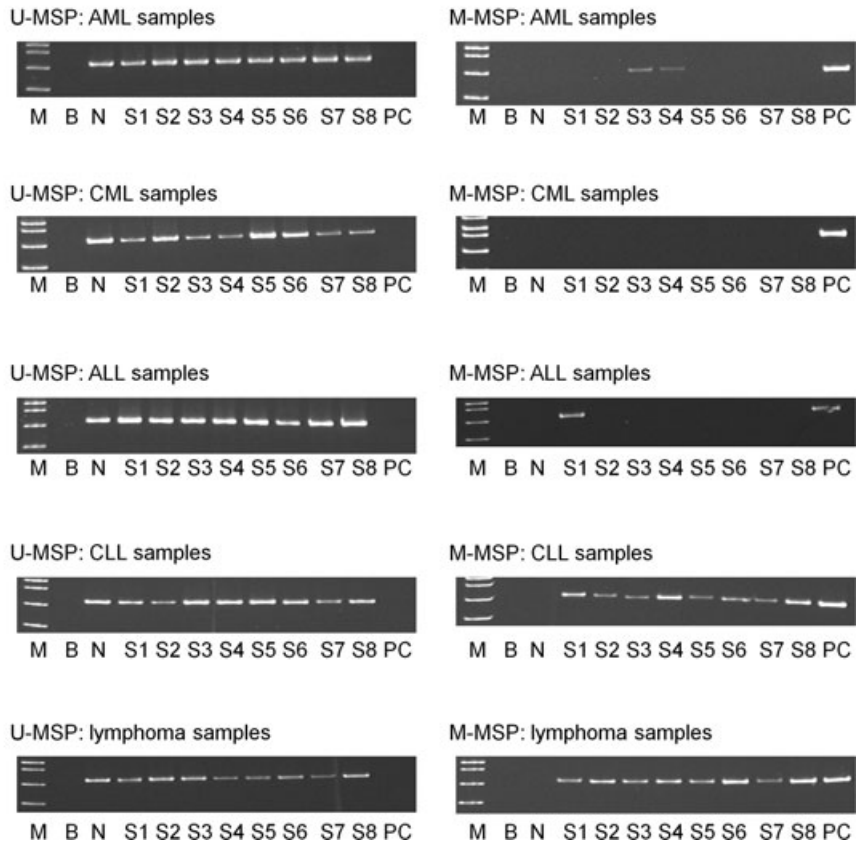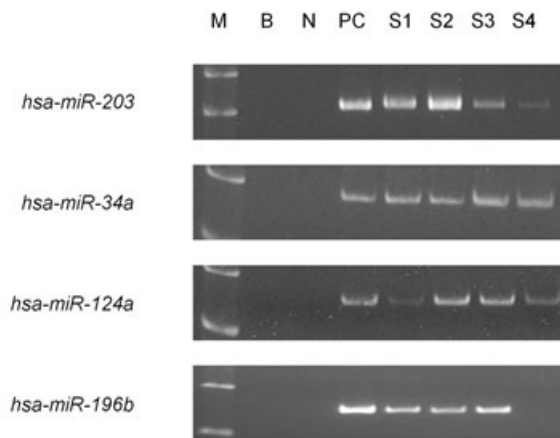
B

U-MSP: AML samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

M-MSP: AML samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

U-MSP: CML samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

M-MSP: CML samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

U-MSP: ALL samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

M-MSP: ALL samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

U-MSP: CLL samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

M-MSP: CLL samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

Fig. 2 Continued.

U-MSP: lymphoma samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

M-MSP: lymphoma samples

M B N S1 S2 S3 S4 S5 S6 S7 S8 PC

C

M-MSP

M B N PC S1 S2 S3 S4

hsa-miR-203

hsa-miR-34a

hsa-miR-124a

hsa-miR-196b

B: reagent blank; N: normal control; S: sample
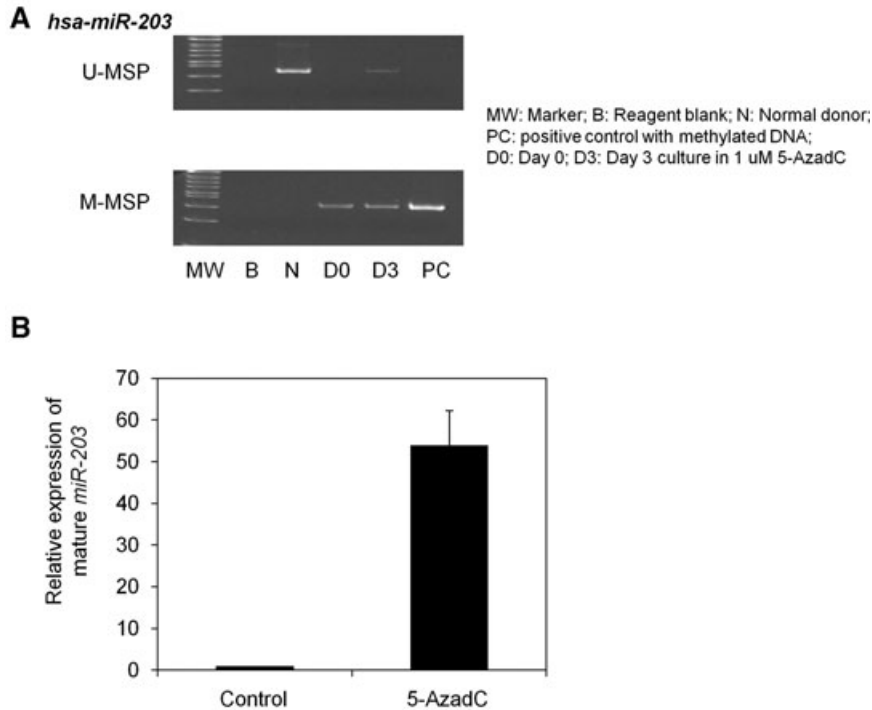
PC: positive control with methylated DNA

Fig. 3 Effect of 5-AzadC treatment on JEKO-1 lymphoma cells. (**A**) M-/U-MSP analysis of *hsa-miR-203* promoter methylation status and (**B**) stem-loop RT-qPCR analysis of the mature *miR-203* expression. 5-AzadC treatment resulted in progressive demethylation of *hsa-miR-203* promoter, and re-expression of the mature *miR-203* in JEKO-1 cells.

5-AzadC treatment of lymphoma cells

JEKO-1 cells were completely methylated for *hsa-miR-203*. Upon 5-AzadC demethylation treatment, *hsa-miR-203* U-MSP signal emerged on day 3 (Fig. 3A), with re-expression of mature *miR-203* as shown by Taqman stem-loop RT-qPCR (Fig. 3B).

Effect of *miR-203* overexpression in lymphoma cells

Upon transfection of mature *miR-203* mimic which led to overexpression mature *miR-203* (Fig. 4A), JEKO-1 showed 20% reduction in cell proliferation as compared with the negative control at 24th hr by MTT assay (Fig. 4B), and 2-fold increase of dead cells as measured by trypan blue exclusion assay (Fig. 4C), thereby suggesting the tumour suppressor role of *miR-203* in lymphoma cells.

Discussion

In this study, we showed that *hsa-miR-203* is hypermethylated and silenced in lymphoma cell lines but not normal blood or marrow cells, which is consistent with a tumour-specific pattern of miRNA-methylation. By contrast, some miRNAs may be hypermethylated in both normal and tumour cells, and hence is consistent with a tissue-specific but not tumour-specific pattern of gene methylation. For example, *miR-127* and *miR-373* are hypermethylated in both the normal and cancer cells [20, 21].

Furthermore, if *miR-203* is a TSG, restoration of *miR-203* expression is expected to inhibit cellular proliferation or induce cell death. Indeed, in this study, restoration of *miR-203* expression in JEKO-1 lymphoma cells led to inhibition of cellular proliferation and enhancement of cell death, which is consistent with previous studies showing inhibition of cell proliferation upon re-expression of *miR-203* in cells carrying homozygous *hsa-miR-203* hypermethylation [12]. Therefore, these findings, together with the pattern of tumour-specific methylation and miRNA silencing, suggested that *miR-203* possesses tumour suppressor property.

In a previous study, *hsa-miR-203* was shown to be preferentially hypermethylated in Ph[+] leukaemias (with expression of BCR-ABL) but less commonly, in Ph[−] acute leukaemias or Ph[−] chronic myeloproliferative diseases [12]. By contrast, we showed that *miR-203* was unmethylated in a Ph[+] CML blastic cell lines, K-562, and also 11 patients with Ph[+] CML at chronic phase. However, the difference might be due to the small number of Ph[+] leukaemia patients in our study. On the other hand, *hsa-miR-203* was preferentially hypermethylated in lymphoid than myeloid malignancies. Moreover, *hsa-miR-203* methylation has been shown to be frequently methylated in T cell lymphoma [12]. In this study, although *hsa-miR-203* methylation was moderately frequent in T cell lymphoma, it was also prevalent in B-cell or NK-cell lymphoma. Therefore, the spectrum of *hsa-miR-203* methylation may be more extensive than Ph[+] neoplasms.
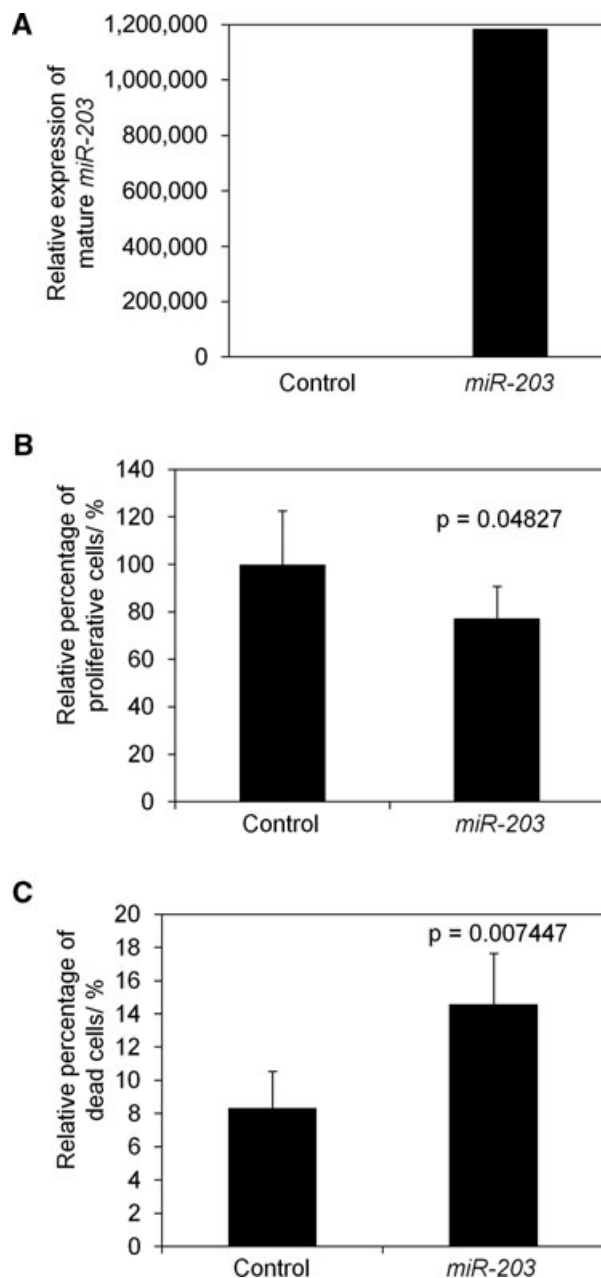
Fig. 4 Effect of *miR-203* in lymphoma cells. JEKO-1 cells, homozygously methylated for *hsa-miR-203*, were transfected with mature *miR-203* mimic oligo as compared with non-targeting precursor control. (**A**) Stem-loop RT-qPCR analysis of mature *miR-203* expression at 24 hrs after transfection. (**B**) Cellular proliferation of lymphoma cells in response to overexpression of *miR-203* was measured by MTT assay. (**C**) Dead cells were measured by trypan blue exclusion assay. Error bars represent standard deviation.

NK/T cell lymphoma is an Epstein–Barr virus-associated, aggressive extranodal lymphoma more frequently encountered in Asia, and Central and South America [22]. As yet, only few TSGs

have been shown to be hypermethylated in NK/T cell lymphoma including *p73, CDKN2A, CDKN2B, hMLH1* and *RARβ* [23]. On the other hand, as yet, *miR-34a* was the only miRNA reported to be hypermethylated in NK/T cell lymphoma [24]. Therefore, frequent methylation of *hsa-miR-203* in NK/T cell lymphoma may be an important biomarker or pathogenetic event.

In our cohort of CLL, despite that it was a retrospective study, survival analysis confirmed that this is a representative CLL with an indolent clinical course, similar to Caucasian CLL patients. Moreover, Rai stage and high-risk karyotypes were important risk factors for survival, thereby testifying that CLL in Chinese runs a similar clinical course as Caucasian CLL patients. However, apart from association with a higher diagnostic Hb level, *hsa-miR-203* methylation did not impact survival.

Finally, although *hsa-miR-203* was frequently methylated in both CLL and NHL, in contrast to CLL in which *hsa-miR-203* was not associated with methylation of other miRNAs, *hsa-miR-203* methylation was associated with methylation of *hsa-miR-34a, -124a* and *-196b*. This finding suggested that different haematological cancers may carry disease-specific miRNA-methylation profiles, which might be important in the pathogenesis of disease.

In summary, *hsa-miR-203* hypermethylation is tumour-specific, associated with gene silencing, which could be reversed by 5-AzadC hypomethylating treatment. *miR-203* possesses tumour suppressor activity. Amongst haematological malignancies, *hsa-miR-203* is more frequently hypermethylated in lymphoid instead of myeloid malignancies. CLL in Chinese patients runs a similarly indolent clinical course, which is impacted by Rai stage and karyotype. Finally, *hsa-miR-203* methylation was associated with concomitant methylation of other miRNAs in NHL.

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There is no financial disclosure.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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