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RNA binding protein MSI2 positively regulates FLT3 expression in myeloid leukemia

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Abstract

FLT3 is frequently mutated and overexpressed in acute myelogenous leukemia (AML) and other hematologic malignancies. Although signaling events downstream of FLT3 receptor tyrosine kinase have been studied in depth, molecular mechanisms of how FLT3 expression is regulated at the post-transcriptional level in particular remain elusive. In this study, we investigated the roles of an RNA binding protein MSI2 as a regulator of FLT3 expression. MSI2 and FLT3 are significantly co-regulated in human AML and chronic myelogenous leukemia in blast crisis (BC-CML). Genetic loss of MSI2 leads to down-regulation of the FLT3 receptor in both AML and BC-CML cells and concomitant impairment of clonogenic growth potential. Furthermore, we demonstrate that MSI2 protein is physically bound to FLT3 mRNA transcripts, suggesting post-transcriptional control of FLT3 expression. Collectively, these results reveal a novel mode of FLT3 regulation essential for leukemia growth, which may aid in designing a targeted therapy to treat human myeloid leukemia.

Keywords

Acute myelogenous leukemia; Chronic myelogenous leukemia; FLT3; RNA binding proteins; Post-transcriptional regulation

1. Introduction

FMS-like tyrosine kinase 3 (FLT3) gene encodes a class III receptor tyrosine kinase (RTK) that plays important roles in the proliferation, differentiation and survival of hematopoietic stem and progenitor cells (HSPCs) [1–3]. FLT3 mutations represent one of the most frequent genetic alterations found in human acute myelogenous leukemia (AML), accounting for greater than 30% of all AML cases [4]. Among those FLT3 mutations, internal tandem duplication (ITD) mutation in the juxtamembrane domain is most common and is clinically significant due to its association with poor prognosis [5–7]. Although wildtype FLT3 RTK requires FLT3-ligand (FL) binding for dimerization and activation, FLT3 with an ITD mutation (FLT3-ITD) is constitutively activated via auto-phosphorylation and thus capable of transmitting proliferative signals independent of FL via multiple downstream pathways, such as STAT5 and PI3K/AKT, for leukemic growth [8–12]. Functionally, FLT3-ITD

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expression alone can induce a fatal myeloproliferative disease that resembles chronic myelomonocytic leukemia in murine models [13–15].

Because of its powerful effect on hematopoietic proliferation and transformation, FLT3 is considered as a promising therapeutic target in AML. To date, several small-molecule inhibitors of the FLT3 tyrosine kinase (FLT3-TKIs) have been developed, and some of them have been successful in blocking AML cell growth in culture and improving overall survival in FLT3-ITD mouse models and clinical trials, indicating that targeting FLT3-mediated signals is an effective strategy to control FLT3-positive AMLs [16–18].

Given that high levels of FLT3 expression, regardless of FLT3 mutational status, have also been reported in greater than 90% of AML and a subset of acute lymphoblastic leukemia (ALL) cases, de-regulation of FLT3 expression may contribute to acute leukemogenesis [19–21]. However, the molecular understanding of how FLT3 expression is regulated, in particular, at the post-transcriptional level remains uncertain. In this study, we investigated potential upstream effector of FLT3 and identified the RNA binding protein MSI2 as a novel regulator of FLT3 expression in myeloid leukemia.

2. Materials and methods

2.1. Murine models of human CML

Mice with BC-CML were generated essentially as previously described [22–24]. In brief, Lin[−] Sca-1⁺ cKit⁺ (LSK) cells were isolated from mouse bone marrow via FACS and retrovirally transduced with the *BCR-ABL1* and *NUP98-HOXA9* oncogenes cloned on MSCV-IRES-YFP or -tNGFR vectors. Infected cells were collected and transplanted into C57BL6/J mice that were preconditioned with sublethal irradiation of 6 Gy. For CP-CML, the *BCR-ABL1* alone is used to transform LSK cells. Following transplantation, mice were maintained on drinking water supplemented with sulfamethoxazole and trimethoprim and evaluated daily for the signs of morbidity, weight loss or anemic appearance. Premorbid animals were sacrificed, and leukemia cells were harvested from hematopoietic tissues and analyzed by flow cytometry or cytology. All mice were 8–16 weeks of age. Msi2 mutant mice, B6;CB-*Msi2*^{Gk(pU-21 T)2Imeg}, was made and established via genetrap mutagenesis at the Center for Animal Resources and Development at Kumamoto University. All animal experiments were performed according to protocols approved by the University of Georgia Institutional Animal Care and Use Committee.

2.2. Viral constructs and production

Retroviral *BCR-ABL1*, *NUP98-HOXA9* and MSCV-IRES-GFP vectors and lentiviral FG12-UbiC-GFP vector were kind gifts from Drs. Warren Pear, Gary Gilliland, Tannishtha Reya and David Baltimore and obtained via Addgene. Lentiviral shRNA constructs were cloned in the FG12 vector as previously described [25]. The target sequences are 5′-CCCAGCAAGTGTAGATAA-3′ for shMSI2-V, 5′-AGTTAGATTCCAAGACGA-3′ for shMSI2-VII and 5′-GGTGACAGCGAATACCTG-3′ for shLacZ as a negative control. The FLAG-HA dual epitope-tagged Msi2 plasmid was constructed by inserting a PCR-generated fragment of mouse Msi2 coding sequence into MSCV-IRES-GFP using Cold Fusion

Cloning strategy (System Biosciences). Virus was produced in 293FT cells transfected using polyethylenimine with viral constructs along with VSV-G and Gag-Pol plasmids. For lentivirus production, a Rev plasmid was also co-transfected. Viral supernatants were collected for two days followed by ultracentrifugal concentration at 50,000g for 2 h.

2.3. Cell culture and colony formation assays

The human leukemia cell lines MV4-11, K562 and THP1 were obtained from ATCC and maintained in Roswell Park Memorial Institute 1640 medium (RPMI-1640) supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin and 100 µg/ml streptomycin. The 293FT cells were maintained in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% FBS, 100 IU/ml penicillin, 100 µg/ml streptomycin and MEM Nonessential Amino Acids Solution. For the colony forming assays, the human leukemia cells were transduced with lentiviral shRNA and plated in triplicate in either 1.2% methylcellulose medium (R&D systems) supplemented with 10% FBS (for MV4-11 and K562) or Human Methylcellulose Complete Media (R&D systems) containing human EPO, GM-CSF, IL-3 and SCF (for THP1).

2.4. Bioinformatics analysis of human gene expression

For the expression patterns of *FLT3* and *MSI2* in normal hematopoietic populations, BloodSpot database was used to retrieve expression data (<http://servers.binf.ku.dk/bloodspot/>) [26]. For the gene correlation analysis, the TCGA dataset LAML (AML) and the GEO datasets GSE4170 (CML), GSE425 (AML) and GSE12417 (CN-AML) were retrieved and analyzed using Python v2.7 and the SciPy statistical toolkit v0.13.3 [27]. Pearson correlation coefficients were used to find patterns of coexpression between *FLT3* and *MSI2* and presented as heatmaps generated using Matplotlib v1.5.0 [28].

2.5. Flow cytometry

Primary leukemia cells from premonitory mice were harvested and treated with ammonium chloride potassium (ACK) buffer to lyse mature red cells. Cells were then blocked with anti-mouse CD16/CD32 (FcBlock) and stained with an antibody cocktail containing 145-2C11 (CD3e), GK1.5 (CD4), 53-6.7 (CD8), RB6-8C5 (Ly-6G/Gr-1), M1/70 (CD11b/Mac-1), TER119 (Ly-76/TER119), 6B2 (CD45R/B220), 2B8 (CD117/c-Kit), D7 (Ly-6A/E/Sca-1) and A2F10 (CD135/Flt3). For human MV-4-11, cells were harvested, blocked and stained with BV10A4H2 for human CD135/FLT3. All antibodies were purchased from eBioscience or Biolegend. Flow cytometric analyses were performed on FACSCanto, MoFlo XDP or CyAn ADP flow analyzers, and data were analyzed with FlowJo software (Tree Star Inc.)

2.6. Western blot

Cells infected with lentiviral shRNA against hMSI2 were collected, washed with cold PBS solution and lysed with the NP40 Lysis Buffer (50 mM Tris/HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% NP-40) supplemented with Halt protease and phosphatase inhibitor cocktails (Thermo Scientific). Cleared cell lysates were resolved by SDS-PAGE, and proteins were electro-transferred to a PDVF membrane (Novex Bolt, Life Technologies). Blots were blocked and probed with anti-MSI2 (EP1305Y, Abcam) or anti-HSP90 (F-8,

Santa Cruz Biotech) followed by HRP-conjugated secondary antibody for chemiluminescence detection (Amersham ECL detection reagent, GE Life Sciences).

2.7. RNA immunoprecipitation (RIP) assays

Mouse leukemia expressing FLAG-HA-tagged Msi2 (FH-Msi2) was generated by infecting primary mouse BC-CML cells with the FH-Msi2-GFP retrovirus. Infected cells were resorted and transplanted in recipient mice as described above. Leukemia cells expressing FH-Msi2 were harvested and stored frozen until use. For RIP assays, frozen leukemia cells were thawed and live cells were sorted. Ten million cells from cell sorts were washed twice with cold PBS and lysed in the NP-40 Lysis Buffer supplemented with protease and phosphatase inhibitors. Human THP1 cells were washed twice with cold PBS and lysed in the NP-40 Buffer. Cell extracts were then subjected to immunoprecipitation with anti-FLAG (M2, Sigma-Aldrich), anti-MSI2 (EP1305Y, Abcam) antibody or a control IgG immobilized on Protein G magnetic beads (Life Technologies) for 1 h at 4 °C. The immunoprecipitated protein-RNA complexes were washed three times each with low- and high-salt wash buffers (300 mM or 550 mM NaCl, respectively) followed by three washes with PBS. Total RNAs in the immunoprecipitated and input fractions were purified using RNAqueous-Micro kit (Ambion) and subjected to RT-PCR analysis for quantification. To quantify RNA amount in each reaction, quantitative real-time PCR was performed using either TaqMan chemistry on an Applied Biosystems 7500 Real-time PCR System or SYBRGreen chemistry on a Biorad iCycler iQ Real-time PCR Detection System according to the manufacturer's instructions. The *Flt3* probe used was Mm00439016 m1. The primers for *hFLT3* were 5'-AGGATGTCAGCTGGCAGATG-3' and 5'-CTACGAATCTTCGACCTGAG-3'.

3. Results

3.1. FLT3 is frequently co-regulated with MSI2 in normal and malignant hematopoiesis

To understand regulation of FLT3 expression, we first performed gene expression analysis using publicly available datasets. In normal human hematopoietic differentiation, *FLT3* expression level strongly correlates with *MSI2* (Fig. 1A and B). MSI2 is a member of MSI family that encodes an RNA binding protein with two tandem RNA Recognition Motifs (RRMs). MSI proteins bind to target mRNAs and post-transcriptionally regulate their expression either positively or negatively [29–31]. Functionally, MSI regulates stem cell self-renewal and differentiation in a variety of mammalian stem cell systems [32–36]. As shown in Fig. 1, both *FLT3* and *MSI2* genes are highly expressed in hematopoietic stem cell population (HSC) and become progressively down-regulated as HSCs differentiate into myeloid progenitors and mature myelocytes. Lymphoid cells express both genes at low levels. Consistent with the expression pattern in normal hematopoietic populations, we found that *FLT3* and *MSI2* are often co-regulated in human myeloid leukemia (Fig. 1C). In a human chronic myelogenous leukemia (CML) dataset, the expression levels for both genes are low in the chronic phase and increase in the blast crisis phase (Dataset GSE4170; p-value = 5.99×10^{-13}). The co-expression pattern is also conserved in acute myelogenous leukemia samples (Datasets TCGA-LAML and GSE425; p-values = 2.4×10^{-3} and 1.1×10^{-2} , respectively). Interestingly, both *FLT3* and *MSI2* are among the gene-expression signature that predicts patient outcome in cytogenetically normal AML (CN-CML) [37]. Consistent

with this previous report, their expressions are significantly co-regulated in CN-AML (Dataset GSE12417; $p\text{-value} = 2.2 \times 10^{-4}$). In addition, we found that AMLs with the FAB subtypes M0/1/2 exhibit a smaller $p\text{-value}$ for the co-regulation pattern than the M3/4/5 subtypes (9.0×10^{-4} for the former group vs. 4.0×10^{-2} for the latter), suggesting that less differentiated myeloblastic leukemias show highly significant correlation in the gene expression. These results suggest a functional link between *FLT3* and *MSI2* in myeloid leukemia.

3.2. Reduced *Flt3* expression by the loss of *Msi2* in murine leukemia

To further examine the role of *MSI2* in *FLT3* expression, we used murine models of CML in the chronic (CP-CML) and blast crisis phase (BC-CML) [38–40]. For the CP-CML model, we isolated normal HSPCs defined by the $\text{Lin}^- \text{Sca-1}^+ \text{cKit}^+$ (LSK) surface marker phenotype from healthy mouse bone marrow via cell sorting, and the LSK cells were transduced retrovirally with the *BCR-ABL1* oncogenes followed by transplantation into pre-conditioned recipient mice. For BC-CML generation, LSK cells were transduced with both the *BCR-ABL1* and *NUP98-HOXA9* oncogenes. The *BCR-ABL1* and *NUP98-HOXA9* oncogenes were identified in human BC-CML cases and induce a fatal leukemia resembling human BC-CML in mouse models [22,41]. Using the leukemia cells from the two models, we first analyzed *Flt3* expression. We have previously shown that *MSI2* is up-regulated in BC-CML [24]. Consistently, we found that BC-CML cells express significantly higher *Flt3*, suggesting that *Flt3* activation is highly conserved between human and mouse BC-CML (Figs. 1 C and 2 A). To examine the contribution of *Msi2* in BC-CML, we next generated BC-CMLs using HSPCs from wildtype (WT) or *Msi2*^{Gt(pU-21 T)2Imeg} mice. In the latter strain, the endogenous *Msi2* gene is disrupted by an insertion of a genetrap vector pU-21T, and the *Msi2* expression in mice homozygous for the mutant allele was reduced to <8% of the WT level in bone marrow [24,42]. The homozygous *Msi2* mutant mice will be referred to as KO animals. Compared with WT control, the BC-CML cells generated from KO cells formed less than half the colonies when plated in cytokine-containing methylcellulose media (Fig. 2B). Within the BC-CML cells, we observed a marked reduction of *Flt3* expression and *Flt3*-positive (*Flt3*⁺) population in leukemic stem/progenitor populations defined by the LSK surface marker phenotype (Fig. 2C). Mean fluorescence intensity of the *Flt3* antibody staining was 335 in WT cells and 136 in KO BC-CML cells. The *Flt3*⁺ population accounted for 45.5% of cells in WT compared with 5.47% in KO. These results suggest that co-regulation of *FLT3* and *MSI2* is conserved both in mouse models and human myeloid leukemia and that *Flt3* down-regulation attenuated clonogenic potential in *Msi2* KO cells.

3.3. shRNA-mediated acute loss of *MSI2* leads to the down-regulation of *FLT3* and impairs leukemic colony formation in human AML with *FLT3*-ITD mutation

Although the above data provided clear genetic evidence for a role of *Msi2* in *Flt3* expression and leukemic growth, it is possible that the germline *Msi2* mutation might cause a developmental defect in hematopoietic cells, which contributed to the impaired clonogenic potential. To exclude this possibility and examine whether *MSI2* can serve as a potential therapeutic target in human AML, we utilized an alternative shRNA-based gene knockdown approach (Fig. 3A). We designed and constructed two lentiviral shRNAs targeting human *MSI2* (shV and shVII). Both constructs successfully knockdown *MSI2* protein expression in

human cells compared with a control shRNA against bacterial LacZ gene (shLacZ), with shVII being more effective in the MSI2 knockdown (Fig. 3B). Next, we infected human MV4-11 FLT3-ITD-positive AML cells with either the MSI2 shRNAs or a control shLacZ and found that this led to a significant reduction in the AML colony formation compared with the control (Fig. 3C; 70% reduction with shVII). Consistent with the data in the murine BC-CML model, we observed a concomitant decrease in the hFLT3 expression in the hMSI2 knockdown cells (Fig. 3D). The MSI2 knockdown with shVII resulted in greater than 90% reduction in FLT3⁺ cells. Importantly, the extent of FLT3 down-regulation correlated with the clonogenic activity, indicating that the regulation of FLT3 by MSI2 is functionally responsible for the growth phenotype in human AML. Interestingly, we also observed impaired colony formation by MSI2 knockdown in human K562 and THP1 cells with wild-type FLT3 genes, suggesting that a functional link between MSI2 and FLT3 regardless of the ITD mutation (Fig. 3E).

3.4. The MSI2 RNA binding protein is physically associated with FLT3 transcripts in leukemia cells

Our findings described above suggest that MSI2 is an upstream regulator of *FLT3* expression and thus AML proliferation. *MSI2* encodes a highly conserved protein with two RRM domains and function as an RNA-binding translational regulator [32,33]. Previous molecular studies demonstrated that MSI1, a paralogue of MSI2 in mammals, binds to an RNA sequence of (A/G)U_nAGU (n = 1–3) [29,43]. Importantly, a recent work by Rentas *et al.* revealed that MSI2 protein binds to a consensus pentamer (G/U)UAGU, indicating a similar sequence specificity to MSI1 [44]. To examine whether *FLT3* is a target of MSI2 protein, we first searched for the consensus MSI2 binding elements (MBEs) in human and mouse *FLT3* genes. Each gene has multiple MBEs across the mRNA from the coding to 3'-untranslated region (UTR; Fig. 4A). Next, we examined whether MSI2 protein is physically associated with *FLT3* mRNAs in leukemia cells by using RNA immunoprecipitation (RIP) assays. We generated BC-CML cells expressing FLAG epitope-tagged mouse Msi2 protein (FLAG-Msi2) and prepared protein extracts of live BC-CML cells for immunoprecipitation with an anti-FLAG antibody. The cellular RNAs co-precipitated with FLAG-Msi2 were purified and reverse-transcribed, and the amount of *FLT3* transcripts was quantified by quantitative real-time PCR. FLAG-Msi2 co-precipitated FLT3 transcripts with a > 79-fold enrichment relative to that of the vector control (Fig. 4B). In order to examine whether the endogenous MSI2 protein is also associated with *FLT3* mRNAs in human leukemia cells, we performed RIP assays in human THP1 AML cells using a specific antibody against human MSI2 protein. Immunoprecipitation with an anti-MSI2 antibody resulted in a significant enrichment of the human *FLT3* transcript compared to a normal IgG control (Fig. 4C), indicating the physical association of MSI2 protein with *FLT3* mRNAs in human AML cells. Collectively, these results demonstrate that MSI2 protein binds to *FLT3* transcripts and positively regulates FLT3 protein expression in myeloid leukemia.

4. Discussion

In this study, we demonstrate that transcript of the FLT3 RTK is a novel mRNA target of the oncogenic RNA binding protein MSI2 in human and mouse myeloid leukemia.

Consequently, genetic loss of *MSI2* resulted in the down-regulation of cell surface FLT3 protein expression and impaired leukemia cell growth.

Activation of FLT3 expression, both at the transcript and protein levels, is frequently observed in human AML as well as in B-ALL and BC-CML cases (Refs. [19,45,46] and Fig. 1C). Therefore, regulatory mechanism of *FLT3* expression constitutes a potential target of transformation events. Consistent with this idea, homeobox transcription factors, such as HOXA9, PBX3 and MEIS1, cooperate to activate *FLT3* mRNA transcription and are capable of inducing *de novo* AMLs when overexpressed [47–51]. The bZIP transcription factor CEBP α and the proto-oncogene c-Myb have also been implicated in the *FLT3* transcriptional activation in AML [52]. These previous studies clearly demonstrated that aberrant *FLT3* activation mediates leukemogenesis triggered by oncogenic transcription factors. Our present study provides evidence for a post-transcriptional control of *FLT3* gene for the first time, which adds a novel regulatory mechanism contributing to the development of human myeloid leukemia.

Given that *FLT3* is most frequently mutated in human AML and the cases with an ITD mutation are significantly associated with adverse clinical outcome, molecular targeting of the FLT3 kinase has become an intense area of research for novel cancer therapeutics [4,5,53]. Several small-molecule FLT3 TKIs, such as AC220/quizartinib, have been evaluated, but the therapeutic effectiveness has been modest in clinics [53,54]. More importantly, resistance to FLT3 TKIs develops through acquisition of point mutations in the kinase domain that interfere with drug binding [55]. In that light, our findings that *MSI2* RNA binding protein regulates FLT3 expression and leukemic growth may provide a new direction in targeted AML therapy. Investigating whether the combined inhibition of *MSI2* and FLT3 functions impair AML development and maintenance *in vivo* would aid in the development of a novel therapeutic approach to treat and cure human hematologic malignancies.

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Fig. 1.

FLT3 is co-regulated with *MSI2* in normal and malignant hematopoiesis. (A) *FLT3* and (B) *MSI2* gene expression levels in normal hematopoietic cell population. Data were retrieved from the BloodSpot database. HSC BM, hematopoietic stem cells from bone marrow (BM); early HPC BM, hematopoietic progenitor cells from BM; CMP, common myeloid progenitors; GMP, granulocyte-monocyte progenitors; MEP, megakaryocyte-erythroid progenitors; PM BM, promyelocytes from BM; MT BM, myelocytes from BM; PMN BM, polymorphonuclear cells from BM; PMN PB, polymorphonuclear cells from peripheral blood (PB); B cells, CD19⁺ B cells; NK cells, CD56⁺ natural killer cells; mDC, CD11c⁺ myeloid dendritic cells; pDC, CD123⁺ plasmacytoid dendritic cells. (C) Heatmap presentations of *FLT3* and *MSI2* expressions in human myeloid leukemia. Expression data of CML patient subsets were retrieved from the NCBI Gene Expression Omnibus database (Accession no. GSE4170). Datasets of AMLs were TCGA-LAML and GSE425, and the CN-AML dataset was GSE12417. CP, CML in chronic phase; AP, CML in accelerated phase; BC, CML in blast crisis phase. M0–M5 indicate FAB subtypes of each group.

Fig. 2.

Impaired colony forming activity and Flt3 expression by the loss of *Msi2*. (A) Flt3 expression in mouse CP- and BC-CML cells. The expression levels are normalized and displayed relative to the expression of the beta-2-microglobulin control. Error bars indicate s.e.m. (n = 3 each), *p < 0.05. (B) Colony-forming potential of *Msi2* mutant BC-CML cells. Live leukemia cells expressing BCR-ABL1 and NUP98-HOXA9 oncogenes with the indicated genotypes were FACS-purified, and 3000 cells were plated per well for colony formation. WT, wildtype and KO, *Msi2* homozygous mutant. *p < 0.05. (C) Flow cytometric analysis of cell surface expression of Flt3 receptor in BC-CML. Murine primary BC-CML cells were stained with a cocktail of antibodies to hematopoietic lineage markers, Sca-1 and c-Kit and Flt3 as described in the Methods. Left panels, dot plots show leukemic stem/progenitor population (LSK) with the indicated genotype. Right panels, histograms show the Flt3 expression levels within LSK population. Each histogram includes an overlay with a Fluorescence Minus One control (FMO) plot in which the Flt3 antibody was omitted when staining.

Fig. 3.

Lentiviral knockdown of human MSI2 leads to reduced FLT3 expression and colony forming activity in human AML. (A) Schema of the structures of human MSI2 proteins. The blue boxes represent RNA Recognition Motifs RRM1 and 2. Sequences targeted with shRNAs in this study (shV and shVII) are shown as bars. (B) MSI2 protein levels in 293FT cells transduced with the indicated lentiviral shRNA constructs. Two independent shRNA constructs, shV and shVII, were used to knockdown hMSI2. Human β -tubulin protein (β -Tub) was used as the loading control. (C) MV4-11 cells infected with the *MSI2* shRNAs lentivirus were plated and assessed for their colony forming activities. Photomicrographs indicate representative colony formed with each condition. shLacZ, a control shRNA for the bacterial *lacZ* gene. * $p < 0.05$. (D) Human MV4-11 AML cells were infected with the indicated lentiviral construct and surface FLT3 expression levels were analyzed via flow cytometry. Bottom panel shows an overlay of all histograms. Fractions of the FLT3⁺ and FLT3⁻ population are shown in each histogram. (E) Colony forming activities of K562 and THP1 cells with *MSI2* knockdown. shLacZ, a control shRNA for the bacterial *lacZ* gene. Error bars indicate s.e.m. * $p < 0.05$, *** $p < 0.001$.

Fig. 4.

The MSI2 RNA binding protein is physically associated with FLT3 transcripts in leukemia cells. (A) Schema of the structures of human and mouse Musashi2 transcript. The yellow boxes represent the coding sequence (CDS) for FLT3 protein. The pink circles represent the putative MSI binding elements (MBEs). (B) RNA immunoprecipitation (RIP) with anti-FLAG tag antibody from BC-CML cells expressing the empty vector (FLAG-empty) or FLAG-tagged Msi2. Co-immunoprecipitated RNAs were purified, reverse transcribed and amplified using primers specific for *Flt3* gene. The values are shown as the fold enrichment of the transcript amounts in the RIP fraction over the amount present in the input sample before RIP (RIP/input). Error bars indicate s.e.m. *** $p < 0.001$. (C) RIP assay with anti-MSI2 antibody in human THP1 AML cells. MSI2-associated RNAs were immunoprecipitated with a specific antibody against endogenous MSI2 proteins (α MSI2) or an IgG control (nIgG). Co-precipitated RNAs were analyzed as described above. Error bars indicate s.e.m. * $p < 0.05$.