UHRF1 regulates the transcriptional repressor HBP1 through MIF in T acute lymphoblastic leukemia

JIE YAO[1], YAOSHENG LUO[1], CHONG ZENG[1], HAIYAN HE[2] and XIAOLI ZHANG[2]

[1]The Department of Laboratory Medicine and Medical Research Center, Shunde Hospital, Southern Medical University (The First People's Hospital of Shunde Foshan), Foshan, Chongqing 528308; [2]Department of Laboratory Medicine, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, P.R. China

Received November 30, 2020; Accepted February 12, 2021

DOI: 10.3892/or.2021.8082

Abstract. Macrophage migration inhibitory factor (MIF) has been confirmed as an oncogene in solid tumor development, and its overexpression causes cell proliferation in T acute lymphoblastic leukemia (T-ALL); however, the underlying mechanisms remain unclear. The overexpression of MIF promotes cellular transformation and proliferation, in part, through interaction with UHRF1. Nevertheless, overexpression of UHRF1 cannot upregulate *MIF* expression in T-ALL. New insights into MIF regulation in T-ALL are imperative to offer the opportunity for therapeutic intervention. In the present study, using RT-qPCR, western blot analysis, confocal microscopy and RNA sequence, we report the identification and validation of UHRF1 as a negative regulator of *MIF*, which functions to downregulate MIF expression by binding to the CATT repeat sequence of the *MIF* promoter. By contrast, HMG-box protein 1 (*HBP1*) functions as a positive regulator of MIF. Moreover, we demonstrated that HBP1 suppressive signaling is reduced by UHRF1 through promotion of the interaction between MIF and HBP1. *MIF* deficiency caused by *UHRF1* knockdown resulted in enhanced apoptosis in T-ALL as compared with that caused by decreased MIF or increased HBP1 expression alone. These results identify UHRF1 as a key regulator of *MIF* transcription in T-ALL, although these transcription factors possess opposite regulatory functions. Thus, this mechanism may provide insight into how to effectively prevent MIF-dependent oncogenic activity. Finally, T-ALL mice possessing high HBP1 or low UHRF1 expression levels are associated with longer survival as compared with control mice, with *UHRF1*-knockdown mice living the longest. Taken together, these findings indicate that MIF and its regulators are potential treatment targets and biomarkers for the prediction of prognosis in T-ALL.

Correspondence to: Dr Jie Yao, Department of Laboratory Medicine and Medical Research Center, Shunde Hospital, Southern Medical University, (The First People's Hospital of Shunde Foshan), 1 Jiazi Road, Foshan, Guangdong 528300, P.R. China
E-mail: jie.yao413@yahoo.com

Abbreviations: UHRF1, inverted CCAAT box binding protein 90 kDa; HBP1, HMG-box protein 1; MIF, macrophage migration inhibitory factor; ALL, acute lymphoblastic leukemia; Co-IP, co-immunoprecipitation; confocal, confocal microscopy

Key words: inverted CCAAT box binding protein 90 kDa, HMG-box protein 1, acute lymphoblastic leukemia, macrophage migration inhibitory factor, regulation

Introduction

Macrophage migration inhibitory factor (MIF) is a regulatory cytokine involved in the immune response, and as such plays an important role in the pathogenesis of autoimmune diseases and cancer (1,2). Findings have shown that CATT repeat number in the *MIF* promoter is associated with MIF expression level. The $CATT_5$ repeat is the lowest expression allele and $CATT_8$ is the highest (3), with the clinical severity of autoimmune inflammatory diseases and immune susceptibility being linked to higher CATT repeats (4). Thetranscription factor UHRF1 (90 kDa inverted CCAAT box-binding protein) can bind to CATT-repeat polymorphisms to regulate MIF expression, and is essential for the $CATT_{5\text{-}8}$ length-dependent regulation of *MIF* transcription (5). UHRF1, as an epigenetic regulator, is overexpressed in cancer and coordinates gene silencing of tumor suppressors (6,7), potentially serving as a biomarker to differentiate among different tumor grades (8).

Evidence suggests that HMG-box protein 1 (HBP1) can bind to the *MIF* promoter and counter-regulate *MIF* expression (9). HBP1 is a member of the high mobility group (HMG) family of transcription factors and has been demonstrated to act as a transcriptional inhibitor in numerous cell lines, with its activation potently inhibiting the cell cycle and regulating related genes (10); therefore, it has been suggested to function as a tumor suppressor. Moreover, *HBP1* maps to chromosome 7q31.1, which has been reported to be frequently deleted in myeloid and other cancers (11,12).

In the present study, we demonstrated that UHRF1 downregulates *MIF* expression by binding to the CATT repeat of the *MIF* promoter, and decreases *HBP1* expression by promoting the interaction between MIF and HBP1 in T acute lymphoblastic leukemia (T-ALL). In addition, HBP1 negatively regulates *MIF* expression as a suppressor in T-ALL, and MIF knockdown prolongs the life of T-ALL mice, suggesting that MIF transcriptional regulation plays an important role in

the pathogenesis of T-ALL and is a potential treatment target and biomarker for the prediction of prognosis in T-ALL.

Materials and methods

Study approval. The Ethics Committee of Shunde Hospital (Fo Shan) approved the use of discarded peripheral blood from T-ALL patients for T-cell cultivation. Informed consent for the procurement and analysis of these samples was also obtained.

Cells and reagents. The human Jurkat T-cell line was purchased from the American Type Culture Collection and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. The apoptotic stains, PI and FITC-Annexin V (BD 556547), were obtained from BD Biosciences. The anti-HBP1 (sc-515281), β-actin (sc-47778), MIF (sc-271631) and anti-UHRF1 (ab57083) antibodies were purchased from Santa Cruz Biotechnology and Abcam, respectively. The Co-IP kit (26149 Pierce) was purchased from Pierce and the EasySep™ Human T Cell Isolation kit was obtained from Stem Cell (cat. no. 17951, Stem Cell).

Western blot analysis. Total protein obtained from Jurkat and PBMC from T-ALL patients was extracted using RIPA lysis and extraction buffer (Thermo Fisher; cat. no. 89900), and equal amounts (Pierce™ BCA Protein Assay, 23227) of 30 μg were resolved in 10% SDS-PAGE at 25 mA for 1 h on ice. The protein bands were subsequently transferred to PVDF (polyvinylidene difluoride) membrane, and non-specific sites were blocked with 5% BSA. The membranes were incubated overnight at 4˚C with an anti-UHRF1 (ab57083, 1/1,000) antibody, washed three times with PBST, and incubated with an HRP-conjugated anti-rabbit secondary (ab205719, 1/2,000) antibody for 2 h at room temperature. ECL detection reagent (Pierce) was used to detect the protein complexes. Densitometric analysis was performed using NIH Image (version 1.62f). The *MIF* promoter $CATT_X$ length-dependent retention of protein was detected by western blotting of eluted binding proteins using an anti-UHRF1 or anti-HBP1 antibody (sc-515281, 1/1,000). The following oligos for $CATT_{0,5-8}$ were used to bind UHRF1: 5'-CTTTCACCCAGCAGTATTAGT CAAT-3' 5'-CTTTCACC*CATTCATTCATTCATTCATT*CA GCAGTATTAGTCAAT-3' 5'-CTTTCACC*CATTCATTCA TTCATTCATTCATT*CAGCAGTATTAGTCAAT-3' 5'-CTT TCACC*CATTCATTCATTCATTCATTCATTCATT*CAGCAGTAT TAGTCAAT-3' 5'-CTTTCACC*CATTCATTCATTCATTCAT TCATTCATTCATT*CAGCAGTATTAGTCAAT-3'

Flow cytometry. PI (propidium iodide) and FITC-Annexin V staining was performed to evaluate apoptosis in Jurkat cells and primary T cells purified from T-ALL patients by Ficoll-Hypaque and a $CD3^+$ T-cell isolation kit following knockdown of UHRF1 or overexpression of HBP1. Staining was analyzed using a FACS Calibur (BD Biosciences).

Immunofluorescence confocal microscopy. PBS-rinsed cultured cells were fixed with methanol on ice for 2 h, blocked with 5% BSA for 1 h, and incubated with the primary antibodies (anti-HBP1, sc-515281; MIF, sc-271631; and anti-UHRF1, ab57083; 1/500) overnight at 4˚C. The following day, the cells were rinsed five times with PBS and incubated with a fluorescently labeled secondary antibody in the dark for 2 h at room temperature. After rinsing, ProLong™ Gold Antifade Mountant with DAPI (P36931) was employed for nuclear staining. Imaging was performed on a Leica YSCC SP5 confocal system at a magnification of x100.

Luciferase reporter assay analysis. MIF-794 $CATT_{5-8}$-dependent transcription was analyzed using the dual luciferase reporter assay system as previously described (5). Each transfection experiment was performed in triplicate and repeated at least twice.

Co-immunoprecipitation. Cells ($1x10^6$) were transfected with empty vector or expression plasmid using Amaxa Nucleofector™. After 24 h, the cells were lysed in IP lysis buffer (Pierce, cat. no. 87787) containing protease inhibitors (Roche), and lysates were centrifuged at approximately 13,000 x g for 10 min to pellet the cell debris at 4˚C and incubated with AminoLink™ Plus coupling resin (Pierce 26149) overnight at 4˚C. The beads were washed three times with IP wash buffer (0.025 M Tris, 0.15 M NaCl, 0.001 M EDTA, 1% NP-40, 5% glycerol, pH 7.4), and the immunoprecipitates were eluted with elution buffer (DTT-containing SDS sample buffer) and boiled for 5 min in SDS loading buffer. Eluates were analyzed by western blot analysis.

Reverse transcription-quantitative PCR. Total RNA of Jurkat and PBMC of T-ALL patients was isolated using an RNeasy RNA extraction kit (Qiagen), and cDNA was synthesized using a BioRad iScript cDNA synthesis kit. RT-qPCR was carried out using the iQ SYBR-Green system (Bio-Rad). Primer sequences were: UHRF1: (5'-ATGTGGATCCAGGTTCGGA-3' and 5'-GAACAG CTCCTGGATCTT-3') and HBP1: (5'-TGAAGGCTGTGATAA TGAGGAAGAT-3' and 5'-CATAGAAAGGGTGGTCCAGCT TA'-3). *MIF* mRNA was determined using the primers: 5'-CGG ACAGGGTCTACATCAA-3', 5'-CTTAGGCGAAGGTGGAG TT-3' and 18S 5'-GCAATTATTCCCCATGAACG-3', 5'-TGT ACAAAGGGCAGGGACTT-3'. The emitted fluorescence for each reaction was measured during the annealing/extension phase and relative quantity values were calculated by the standard curve method. The quantity value of 18S in each sample was used as a normalizing control. Differences were evaluated by non-parametric testing using the Mann-Whitney U test.

Jurkat and primary T-cell transfection. Primary T cells were isolated by EasySep™ Human T Cell Isolation Kit (Stem Cell, cat. no. 17951), then transfected using Nucleofector™ solution (Lonza, cat. no. VPA-1002) and the Nucleofector™ II system for transfecting T cells. After 24 h in an incubator at 37˚C, the cells were harvested for further experimentation. The shRNA plasmids included UHRF1 (GI333964), HBP1 (TL312507), MIF (TR319111), or control (TR30007). The overexpression plasmid was HBP1 (RG202260) or control (PS100010) (Origene).

In vivo leukemia cell transplantation. NOD-SCID-γ (NSG) mice (8-10 weeks old) were obtained from Biocytogen (Beijing) provided with autoclaved food and clear H_2O and housed in a specific pathogen-free (SPF) facility. Animal care was carried out in accordance with the local Animal Welfare Act. All food, water, bedding, and cages within the room were autoclaved or sterilized and cages were changed weekly; the

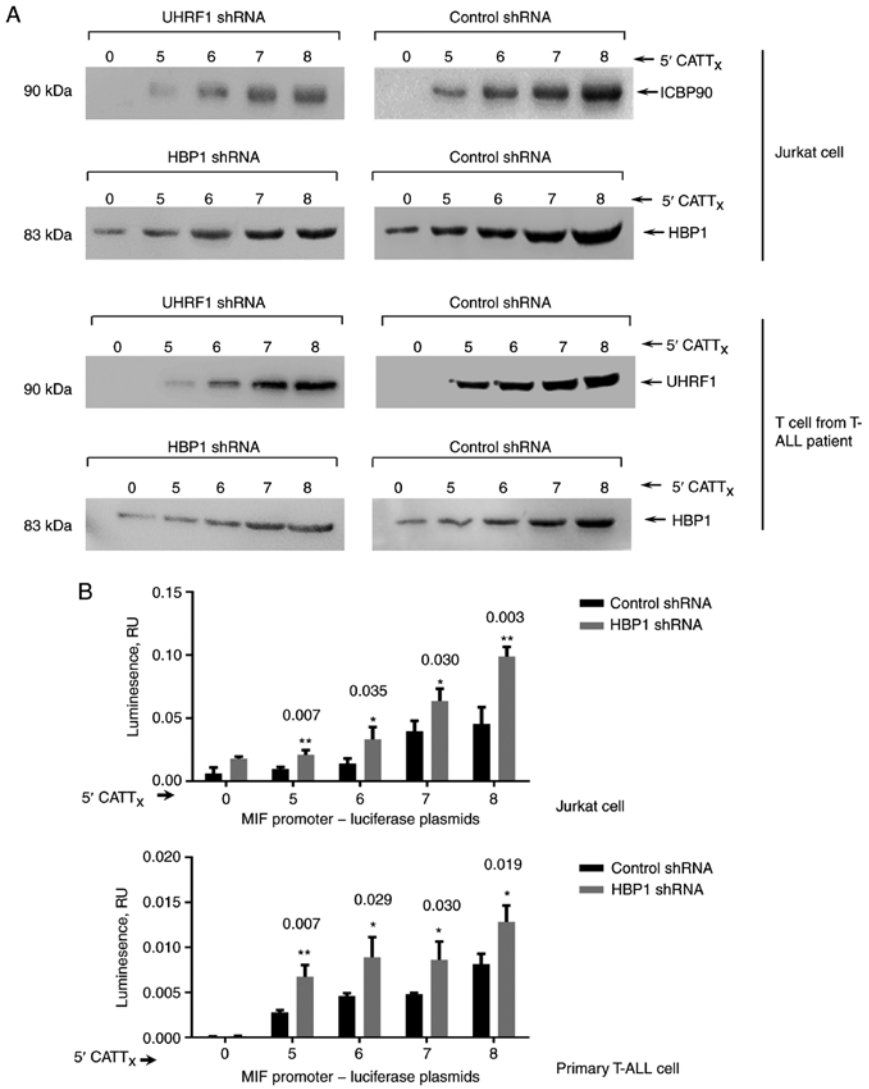

Figure 1. (A) UHRF1 specifically binds to the CATT polymorphism of MIF but HBP1 does not. Western blot analysis reveals that UHRF1 binds to the CATT motif in the MIF promoter in a CATT length-dependent manner in Jurkat and primary T cells. HBP1, not only binds to promoter sequences including CATT, but also to those excluding CATT, indicating that HBP1 does not specifically bind to the CATT polymorphism. Jurkat or primary T cells were cultured in 6-well plates ($1x10^6$ cells/well) and transfected with UHRF1 or control shRNA for 48 h. DNA-bound UHRF1 and HBP1 were detected following incubation of nuclear lysates with 100 nM biotin-labeled 5' $CATT_{0-8}$ oligonucleotides spanning the MIF promoter (-865/-833 to -752). The 5' $CATT_{0-8}$ oligonucleotide-bound proteins were captured by streptavidin-bead absorption after a 3-h incubation at 4˚C, and 1 mg was separated by SDS-PAGE and immunoblotted with anti-UHRF1 and anti-HBP1 antibodies. (B) HBP1 knockdown stimulates MIF expression. T cells were transfected with *MIF* promoter-luciferase reporter plasmids, treated with HBP1 or control shRNA, and cultured for 24 h. Prior to measurement of luciferase activity, cells were analyzed under basal conditions. Data are expressed as the mean ± SD of three measurements repeated twice (n=three measurements per experiment). **P<0.01 and *P<0.05 for control shRNA vs. HBP1 shRNA (Student's t-test, two-tailed).

room temperature was 26-28˚C, and humidity was kept at 40-60%; with a 12-h light/dark cycle. A total of 36 mice were injected via the tail vein with $1x10^6$ cells per mouse (T-ALL cells transfected with UHRF1-shRNA, HBP1-overexpression, or scrambled shRNA control plasmids). For survival experiments, mice (n=12 mice per group) were culled 25 days post-engraftment, or immediately following the appearance of signs of moribund or weight loss exceeding 10-15% of their total weight.

Statistical analysis. Results are expressed as the mean ± standard deviation. To study the difference between two groups, the Student's t-test and approximate calculation of normal distribution were used for all two-tailed comparisons. One-way ANOVA followed by Tukey's post-hoc test was used to compare more than two groups. The similarity of expression levels in the transcriptome was assessed by Pearson's correlation analysis. Expression heat map of genes were selected for 1.5-fold differential expression with an FDR <0.05 to show the different genes associated with MIF between ALL and healthy control. NSG mice survival was assessed via Kaplan-Meier survival curve. Analyses were performed using the GraphPad Prism software. P<0.05 was considered statistically significant, and P<0.01 was considered statistically very significant.

Results

Identification of HBP1 and UHRF1 binding to the MIF promoter and regulation of MIF transcription. To investigate whether HBP1 and UHRF1 can interact with the MIF-794 $CATT_{5-8}$ microsatellite in T-ALL, 5' biotin-labeled oligonucleotides, including or excluding CATT repeats of the *MIF* promoter

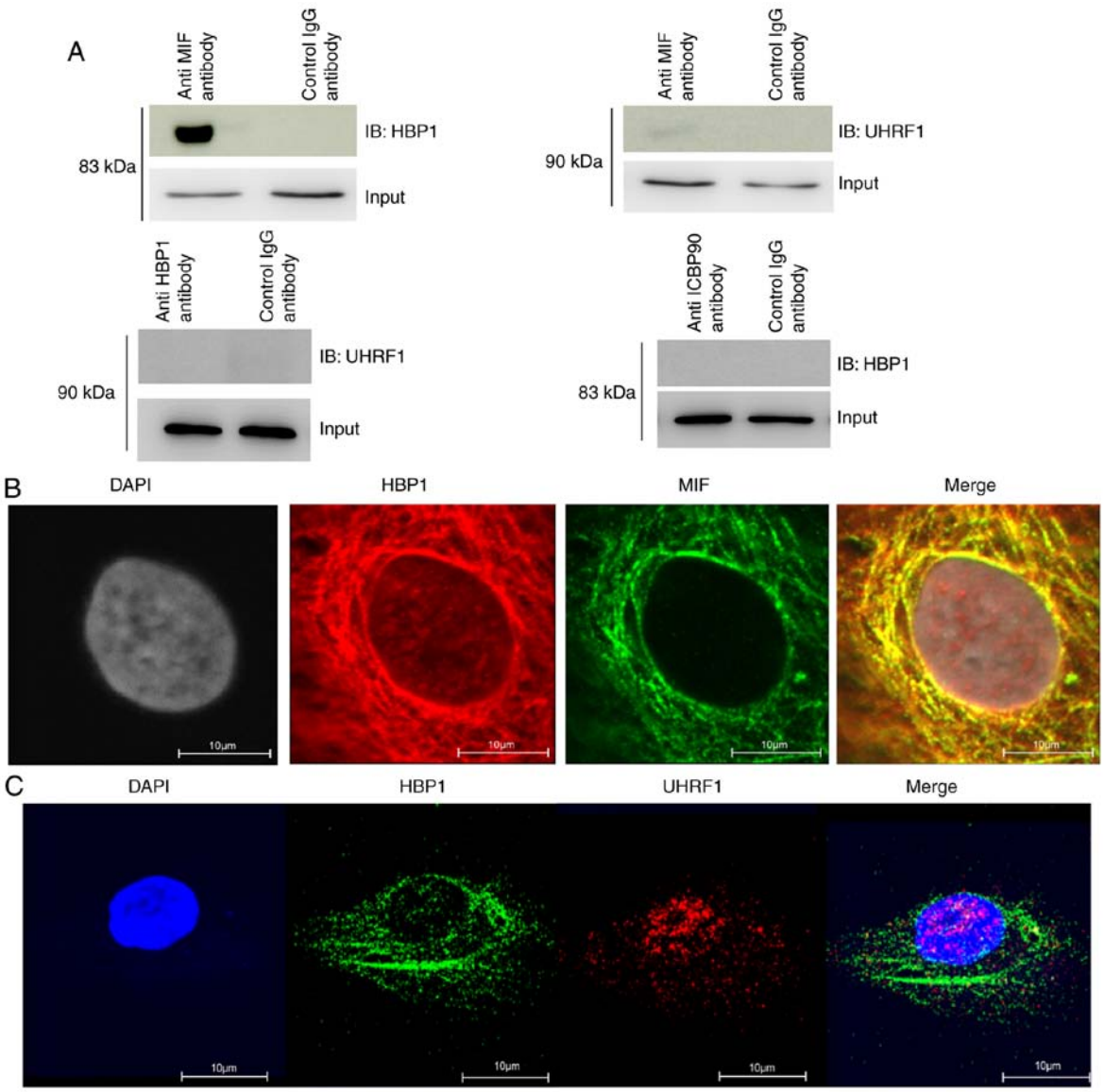

Figure 2. Protein interaction between MIF and HBP1 in the cytosol. (A) Co-immunoprecipitation and immunoblotting showing protein interaction. Jurkat cells were cultured in 6-well plates (1x10^6 cells/well) and lysed in IP lysis buffer containing protease inhibitors. Lysates were centrifuged and incubated with AminoLink coupling resin (Pierce 26149) and anti-UHRF1, anti-HBP1, or anti-MIF antibodies for at least 4 h. The beads were subsequently washed four times with cold wash buffer and eluted with DTT-containing SDS sample buffer by boiling for 10 min. Protein samples were prepared in loading buffer containing 0.125 mM Tris-HCl (pH 6.8), 30% (v/v) glycerol, and 2% (w/v) deoxycholate and subjected to SDS-PAGE at 25 mA for 1 h on ice. (B) Confocal microscopy of UHRF1 and HBP1 in HeLa cells. (C) Magnification, x100. UHRF1 localizes mainly to the nucleus but HBP1 localizes to both the nucleus and the cytosol in HeLa and Jurkat cells. HBP1 (green) and UHRF1 (red).

(without any CATT sequences as a control), were incubated with nuclear lysates of human T cells followed by streptavidin beads. NaCl-eluted bound proteins were evaluated by western blot analysis (Fig. 1A). The effectiveness of this approach was verified by testing the CATT-specific interaction of UHRF1 in T cells. As shown in Fig. 1A, the analysis revealed binding of HBP1 to the *MIF* $CATT_8$ and control $CATT_0$ oligonucleotides. We previously demonstrated a downregulatory role of UHRF1 in-794 $CATT_{5-8}$-dependent *MIF* expression (5); thus, we assessed the functional role of HBP1 in *MIF* expression by measuring the transcriptional activity of *MIF* in human T-ALL cells using a luciferase reporter assay. The level of *MIF* promoter transcript increased progressively with increasing levels of HBP1 shRNA, with $CATT_5$ showing the lowest gene transcription and $CATT_8$ showing the highest one (Fig. 1B).

Protein crosstalk between the transcription factor HBP1 and MIF. Co-immunoprecipitation (Co-IP) in T cells verified the interaction between HBP1 and MIF, but not between UHRF1 and HBP1 or between UHRF1 and MIF (Fig. 2A). Subsequently, the location of the interaction between HBP1 and MIF was confirmed in HeLa cells by confocal microscopy, showing co-localization in the cytosol but not in the nucleus (Fig. 2B). Moreover, there was no co-localization of UHRF1 and HBP1 (Fig. 2C).

UHRF1 downregulates both MIF and HBP1 expression. Given that UHRF1 regulates *MIF* expression by binding to *MIF* CATT motifs, and HBP1 acts as a suppressor, we focused further attention on defining the relationship among the three genes. Following knockdown of *UHRF1*, *HBP1*, or both genes simultaneously, RT-qPCR results showed that UHRF1, not only

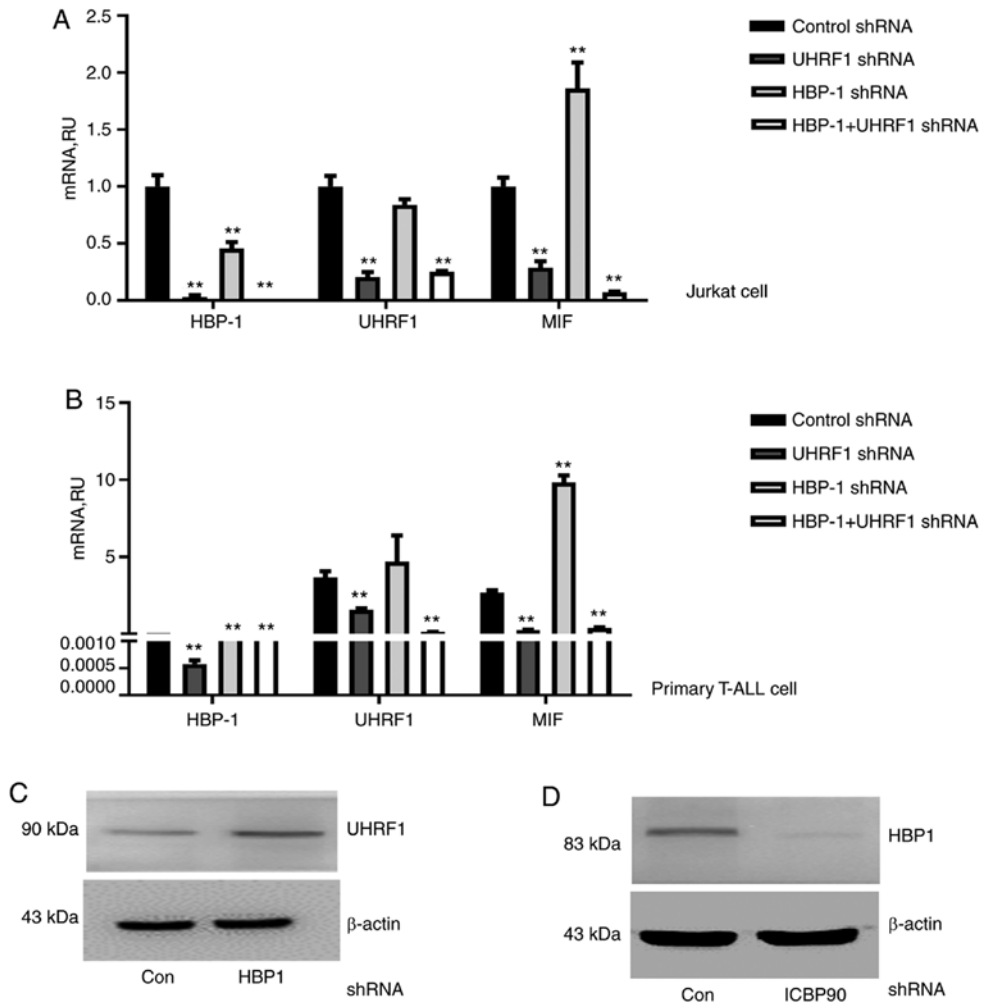

Figure 3. UHRF1 downregulates both MIF and HBP1 in T-ALL cells. (A) RT-qPCR showing that HBP1 knockdown alone can increase MIF expression but UHRF1 knockdown can decrease both MIF and HBP1 expression in Jurkat cells. (B) RT-qPCR showing that HBP1 knockdown alone can increase MIF expression but UHRF1 knockdown can decrease both MIF and HBP1 expression in primary T cells from ALL patients (n=3). (C) Intracellular UHRF1 protein level following HBP1 knockdown as analyzed by western blotting of cell lysates. β-actin served as a loading control. UHRF1 protein expression levels were no different following HBP1 knockdown. (D) Lower HBP1 protein levels were observed following treatment with UHRF1 shRNA. Data are expressed as the mean ± SD of three measurements repeated twice (n=three measurements per experiment). **P<0.01, by Student's t-test (two-tailed). Displayed blots are representative of three independent experiments.

downregulated *MIF* expression, but also *HBP1* expression, causing loss of HBP1 repressive function in both the T-ALL cell line and T cells from ALL patients (Fig. 3A and B). It was further confirmed by western blotting that UHRF1 can downregulate HBP1 at the protein level (Fig. 3C and D). Subsequently, the effect of the overexpression of UHRF1 and HBP1 on MIF regulation was assessed. The MIF expression level was not significantly different following upregulation of UHRF1 or HBP1 (Fig. 4A and B), indicating that UHRF1 cannot upregulate *MIF* and HBP1 cannot downregulate *MIF* in T-ALL. The influence of the interaction between MIF and HBP1 on the downregulation of *HBP1* mediated by UHRF1 was further evaluated. Knockdown of *MIF* by MIF shRNA increased *HBP1* expression in T-ALL cells, which was also observed following *UHRF1* knockdown (Fig. 5A and B). Moreover, UHRF1 knockdown could not downregulate *HBP1* following inhibition of the MIF protein using an inhibitor (Fig. 5C).

MIF silencing induces cell apoptosis and slows leukemia progression in vivo. We also examined cell apoptosis and animal survival following the regulation of MIF expression by UHRF1 and HBP1, which induced apoptosis, retarded the progression of ALL, and extended survival time. As expected, knockdown of *UHRF1* or overexpression of HBP1 in T-ALL cells reduced basal MIF expression (Fig. 6A). MIF overexpression is known to inhibit apoptosis in many cell types, and the two mediators act in concert to regulate apoptotic sensitivity in the context of inflammatory activation (10-13). Experimental reduction of UHRF1 or upregulation of HBP1 enhanced T-ALL cell sensitivity to apoptosis, which is consistent with the interpretation that functional UHRF1 and HBP1 regulate MIF expression and protect cells from apoptosis (Fig. 6B). To further assess the functions of UHRF1 and HBP1 in the progression of T-ALL *in vivo*, mice were injected with transduced Jurkat cells to observe survival time. The results show that mice transplanted with *UHRF1*-knockdown cells lived longer than those in both the control and HBP1-overexpression groups, which is consistent with the cell apoptosis data (Fig. 6C).

Pathogenic role of MIF. Human genetic studies indicate a high expression of *MIF* and *UHRF1* and low expression of *HBP1* in T-ALL, and experimental data suggest a pathogenic role

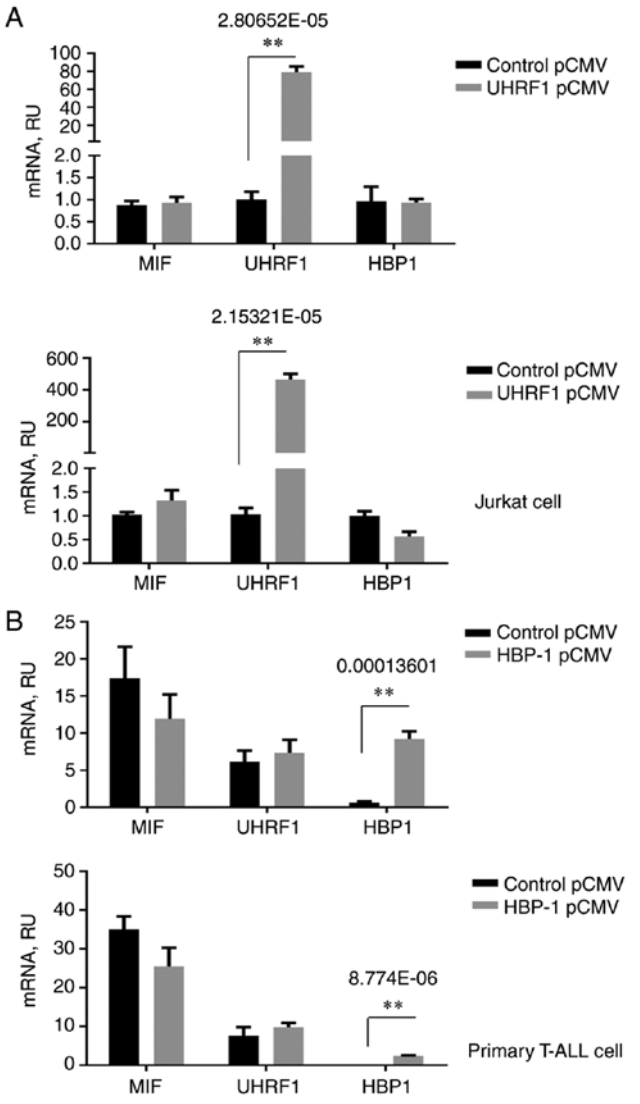

Figure 4. UHRF1 cannot upregulate either MIF or HBP1, and HBP1 overexpression does not downregulate MIF in T-ALL cells. (A) RT-qPCR showing that increased UHRF1 cannot increase MIF or HBP1 expression in Jurkat cells. (B) RT-qPCR showing that HBP1 upregulation cannot decrease MIF expression in primary cells from T-ALL patients. Data are expressed as the mean ± SD of three measurements repeated twice (n=three measurements per experiment). **P<0.01, by Student's t-test (two-tailed). Displayed blots are representative of three independent experiments.

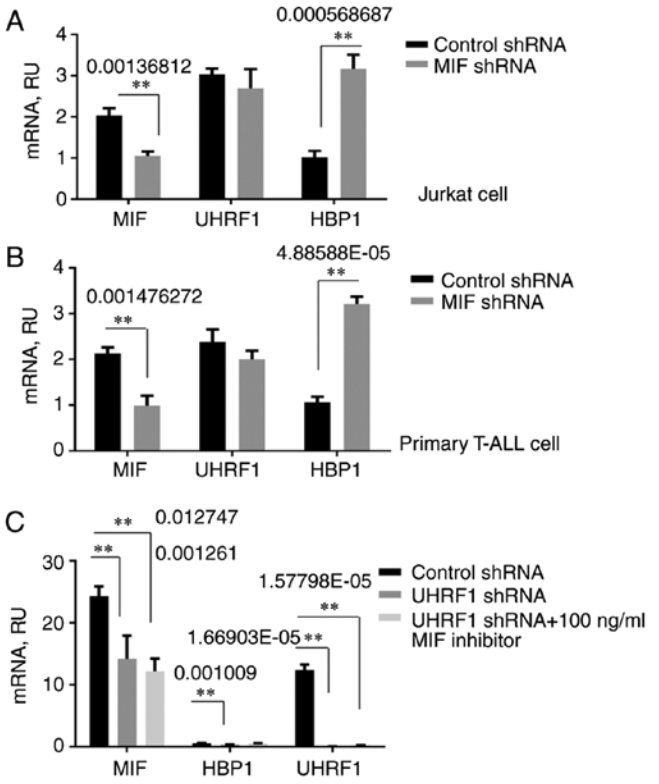

Figure 5. UHRF1 regulates HBP1 expression by promoting the interaction between HBP1 and MIF in T-ALL cells. (A) RT-qPCR showing that MIF negatively regulates HBP1 expression, but not UHRF1 expression, following MIF knockdown in Jurkat cells. (B) RT-qPCR showing that MIF negatively regulates HBP1 expression, but not UHRF1 expression, following MIF knockdown in cells from T-ALL patients (n=3). (C) UHRF1 knockdown cannot decrease HBP1 expression in the absence of MIF in cells from T-ALL patients. Data are expressed as the mean ± SD of three measurements repeated twice (n=three measurements per experiment). **P<0.01, by Student's t-test (two-tailed). Displayed blots are representative of three independent experiments.

of MIF in promoting proliferation and downstream expression of chemokines in T-ALL (Fig. 7A). Notably, a significant correlation was observed between the mRNA expression levels of *UHRF1* and *MIF* (R=0.9192, P<0.0001) and *HBP1* and *MIF* (R=0.6977, P<0.0001) in the T-ALL group as compared with those in the healthy control group (Fig. 7B). This correlation between the expression levels of UHRF1 and MIF and between HBP1 and MIF supports a functional role of UHRF1 downregulation and HBP1 upregulation with respect to MIF in T-ALL.

Discussion

Macrophage migration inhibitory factor (MIF) has been suggested to be a pro-tumorigenic factor that promotes the proliferation, migration, and invasion of tumor cells (13). Previous findings have shown that ALL cells constitutively express high levels of MIF (14). Leukemic cells from most patients express the chemokine IL-8 and the receptor CXCR1, but at lower levels. Moreover, one report used a mouse model in which subcutaneous ALL tumors were partially suppressed by locally injected endothelial IL-8 (15-17). In the present study, T cells were isolated from healthy controls and T-ALL patients and subjected to microarray. These data are consistent with reports that MIF expression is high and causes increased expression of downstream chemokines such as IL-8, which are associated with cell proliferation, suggesting that MIF plays a pathogenic role in T-ALL. Our mechanistic understanding of the MIF-mediated regulation of tumor cell proliferation has expanded since the identification of MIF transcription. Compelling evidence suggests that MIF overexpression and regulation is associated with, and contributes to, the pathogenesis of inflammatory autoimmune and malignant diseases (18,19); however, the mechanism underlying MIF regulation in T-ALL has yet to be clarified.

Evidence suggests that UHRF1 positively regulates *MIF* transcription (5) and HBP1 has a negative regulatory function (9). To determine the key regulatory mechanism of MIF in T-ALL, we identified that UHRF1 and HBP1 co-regulate MIF expression. Of note, UHRF1 can also regulate HBP1 transcription by promoting the interaction between MIF and

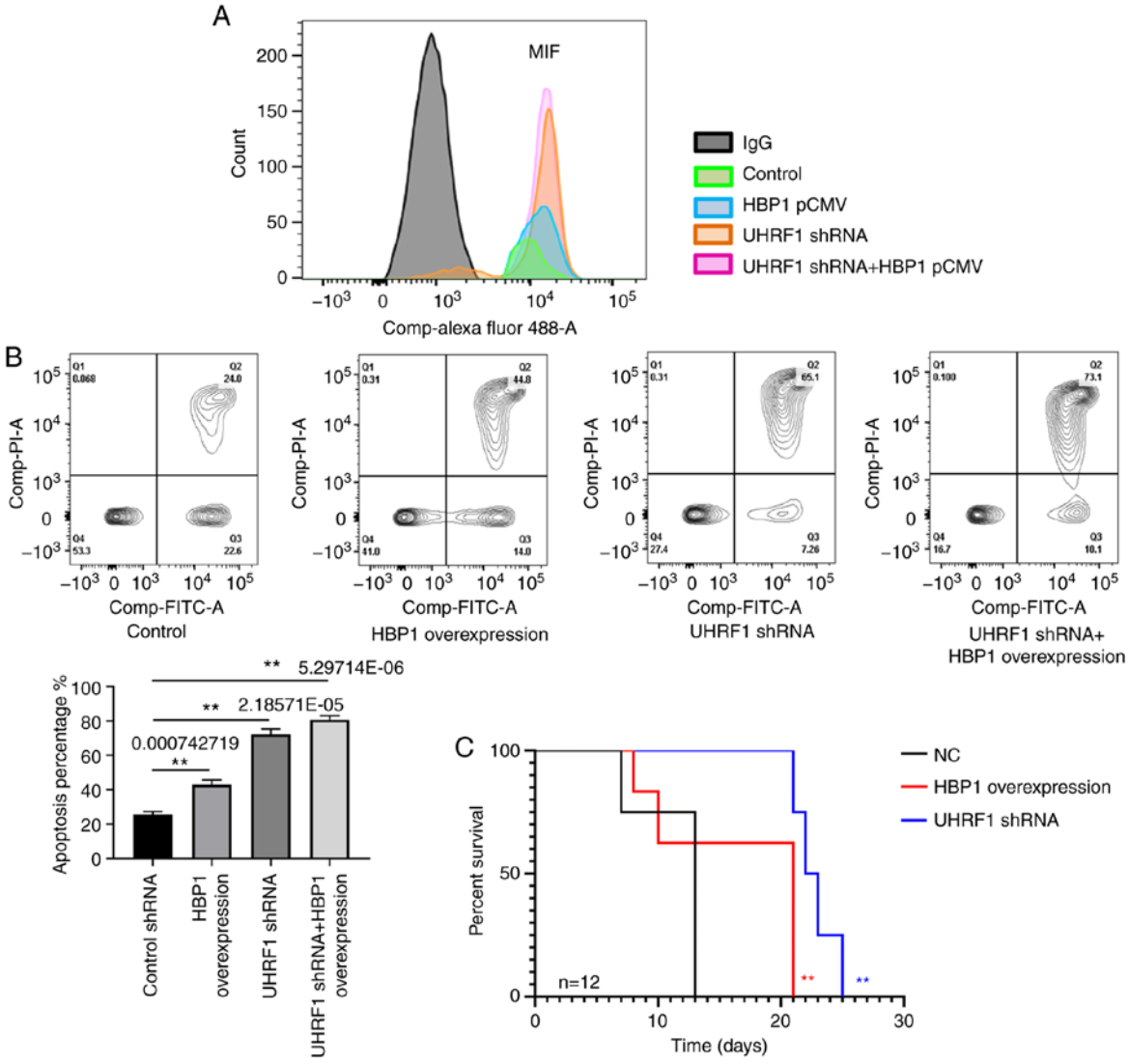

Figure 6. MIF knockdown by its regulators causes apoptosis and significantly increases survival *in vivo*. (A) Primary human T-ALL cells extracted using a lymphocyte separation kit were transfected with control, UHRF1 shRNA, or HBP1 overexpression plasmids for 24 h. Cells were subsequently washed once with PBS, stained with apoptotic markers, and subjected to FACS analysis. Right lower quadrant: early apoptotic cells; left upper quadrant: dead cells; left lower quadrant: live cells; right upper quadrant: late apoptotic cells. All apoptosis experiments were carried out at least three times. One representative experiment is shown. (B) Peripheral blood leukocytes were isolated from T-ALL patients (n=3) and transfected with control, UHRF1 shRNA, or HBP1 overexpression plasmids for 24 h. Cells were subsequently harvested, and the intracellular MIF was measured by flow cytometry. Data represent three individuals per group. **P<0.01 unpaired t-test. (C) Kaplan-Meier survival curve of NSG mice transplanted with HBP1, UHRF1, or empty vector-transfected Jurkat cells. **P<0.01 (log-rank test as compared with the control group).

HBP1 proteins. We verified a specific association between MIF and HBP1 by co-immunoprecipitation of MIF-HBP1 complexes *in vitro*. To confirm that MIF can interact with intracellular HBP1, we showed the co-localization of endogenously expressed MIF and HBP1 in the cytosol. We also observed that UHRF1 can only regulate MIF, but not HBP1 without the presence of MIF. Moreover, HBP1 was able to only upregulate *MIF* expression but could not downregulate *MIF* expression when HBP1 expression was elevated, indicating that UHRF1 is the key regulator in the knockdown of *MIF* in T-ALL.

Furthermore, we found that the expression levels of *UHRF1* and *MIF* were elevated but that of *HBP1* was decreased in T-ALL patients as compared with those in healthy controls. In addition, analysis of the correlation among *UHRF1*, *MIF*, and *HBP1* expression in a gene expression dataset of T cells from T-ALL patients shows a high correlation between *UHRF1* and *MIF* expression and between *HBP1* and *MIF* expression, supporting a positive regulatory role of UHRF1 and a negative regulatory role of HBP1 in MIF transcription *in vivo*. Moreover, UHRF1 knockdown and HBP1 overexpression induced a greater level of apoptosis in T cells from T-ALL patients and significantly prolonged the survival time of transplanted mice.

Taken together, our results indicate an important role of the UHRF1 protein in the survival and homing of malignant T cells, which is mediated through a functional interaction between MIF and HBP1. In conclusion, a high level of UHRF1 and a low level of HBP1 cause *MIF* overexpression, resulting in tumor cell proliferation and inhibition of cell death. The MIF/UHRF1/HBP1 axis may represent a novel target for the therapeutic intervention of ALL.

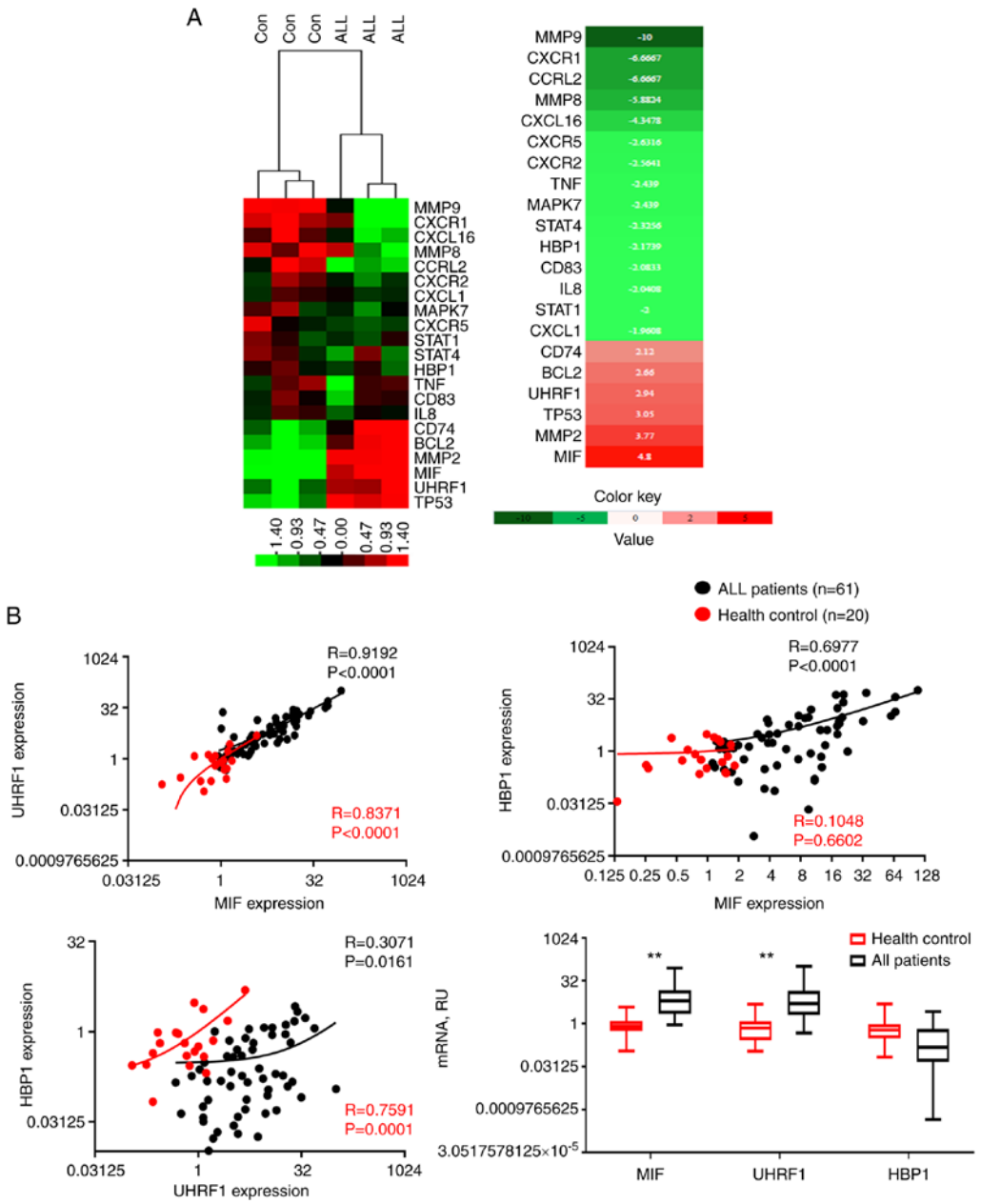

Figure 7. (A) The difference in expression of UHRF1, HBP1, MIF, and MIF-related cytokines between T-ALL patients and healthy controls. Expression heat map of genes selected for 1.5-fold differential expression with an FDR <0.05. Data represent three healthy individuals in comparison with three patients with T-ALL cells. (B) Correlation between UHRF1 and MIF and between HBP1 and MIF, but not between UHRF1 and HBP1 in ALL. Correlation plots for UHRF1 vs. MIF, UHRF1 vs. HBP1, and HBP1 vs. MIF mRNA expression in RNA samples obtained from patients with ALL (n=66) or healthy controls (n=20). The expression scores were calculated from the mean of the normalized values. P<0.0001 for the mean MIF expression in ALL patients (12.81±0.29) vs. healthy controls (9.37±0.15) by a two-tailed Student's t-test. **P<0.01 unpaired t-test.

Acknowledgements

Not applicable.

Funding

We would like to thank the National Natural Scientific Foundation of China (81770148, to J. Yao) for funding.

Authors' contributions

JY contributed to the conception of the study. JY and CZ analyzed data for the study. JY, CZ, HH, YL and XZ performed the experiments for the study. JY and YL wrote the study. All authors approved the study, and JY and YSL confirm the authenticity of the data.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

The Ethics Committee of Shunde Hospital (Fo Shan) approved the use of discarded peripheral blood from T-ALL patients for

T-cell cultivation. Informed consent for the procurement and analysis of these samples was also obtained.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kang I and Bucala R: The immunobiology of MIF: Function, genetics and prospects for precision medicine. Nat Rev Rheumatol 15: 427-437, 2019.
2. Bloom J, Sun S and Al-Abed Y: MIF, a controversial cytokine: A review of structural features, challenges, and opportunities for drug development. Expert Opin Ther Targets 20: 1463-1475, 2016.
3. Liu A, Bao F and Voravuthikunchai SP: CATT polymorphism in MIF gene promoter is closely related to human pulmonary tuberculosis in a southwestern China population. Int J Immunopathol Pharmacol: May 29, 2018 (Epub ahead of print). doi: 10.1177/2058738418777108
4. Matia-García I, Salgado-Goytia L, Muñoz-Valle JF, García-Arellano S, Hernández-Bello J, Salgado-Bernabé AB and Parra-Rojas I: Macrophage migration inhibitory factor promoter polymorphisms (-794 CATT 5-8 and -173 G>C): Relationship with mRNA expression and soluble MIF levels in young obese subjects. Dis Markers 2015: 461208, 2015.
5. Yao J, Leng L, Sauler M, Fu W, Zheng J, Zhang Y, Du X, Yu X, Lee P and Bucala R: Transcription factor ICBP90 regulates the MIF promoter and immune susceptibility locus. J Clin Invest 126: 732-744, 2016.
6. Mousli M, Hopfner R, Abbady AQ, Monté D, Jeanblanc M, Oudet P, Louis B and Bronner C: ICBP90 belongs to a new family of proteins with an expression that is deregulated in cancer cells. Br J Cancer 89: 120-127, 2003.
7. Abbady AQ, Bronner C, Trotzier MA, Hopfner R, Bathami K, Muller CD, Jeanblanc M and Mousli M: UHRF1 expression is downregulated in apoptosis-induced Jurkat cells. Ann N Y Acad Sci 1010: 300-303, 2003.
8. Ashraf W, Ibrahim A, Alhosin M, Zaayter L, Ouararhni K, Papin C, Ahmad T, Hamiche A, Mély Y, Bronner C and Mousli M: The epigenetic integrator UHRF1: On the road to become a universal biomarker for cancer. Oncotarget 8: 51946-51962, 2017.
9. Chen YC, Zhang XW, Niu XH, Xin DQ, Zhao WP, Na YQ and Mao ZB: Macrophage migration inhibitory factor is a direct target of HBP1-mediated transcriptional repression that is over-expressed in prostate cancer. Oncogene 29: 3067-3078, 2010.
10. Bollaert E, Johanns M, Herinckx G, de Rocca Serra A, Vandewalle VA, Havelange V, Rider MH, Vertommen D and Demoulin JB: HBP1 phosphorylation by AKT regulates its transcriptional activity and glioblastoma cell proliferation. Cell Signal 44: 158-170, 2018.
11. Bollaert E, de Rocca Serra A and Demoulin JB: The HMG box transcription factor HBP1: A cell cycle inhibitor at the crossroads of cancer signaling pathways. Cell Mol Life Sci 76: 1529-1539, 2019.
12. Li H, Wang W, Liu X, Paulson KE, Yee AS and Zhang X: Transcriptional factor HBP1 targets P16(INK4A), upregulating its expression and consequently is involved in Ras-induced premature senescence. Oncogene 29: 5083-5094, 2010.
13. Fukaya R, Ohta S, Yaguchi T, Matsuzaki Y, Sugihara E, Okano H, Saya H, Kawakami Y, Kawase T, Yoshida K and Toda M: MIF maintains the tumorigenic capacity of brain tumor-initiating cells by directly inhibiting p53. Cancer Res 76: 2813-2823, 2016.
14. Sharaf-Eldein M, Elghannam D, Elderiny W and Abdel-Malak C: Prognostic implication of MIF gene expression in childhood acute lymphoblastic leukemia. Clin Lab 64: 1429-1437, 2018.
15. Kumar S, O'Malley J, Chaudhary AK, Inigo JR, Yadav N, Kumar R and Chandra D: Hsp60 and IL-8 axis promotes apoptosis resistance in cancer. Br J Cancer 121: 934-943, 2019.
16. Kuett A, Rieger C, Perathoner D, Herold T, Wagner M, Sironi S, Sotlar K, Horny HP, Deniffel C, Drolle H and Fiegl M: IL-8 as mediator in the microenvironment-leukaemia network in acute myeloid leukaemia. Sci Rep 5: 18411, 2015.
17. Vijay V, Miller R, Vue GS, Pezeshkian MB, Maywood M, Ast AM, Drusbosky LM, Pompeu Y, Salgado AD, Lipten SD, *et al*: Interleukin-8 blockade prevents activated endothelial cell mediated proliferation and chemoresistance of acute myeloid leukemia. Leuk Res 84: 106180, 2019.
18. Bilsborrow JB, Doherty E, Tilstam PV and Bucala R: Macrophage migration inhibitory factor (MIF) as a therapeutic target for rheumatoid arthritis and systemic lupus erythematosus. Expert Opin Ther Targets 23: 733-744, 2019.
19. Noe JT and Mitchell RA: MIF-dependent control of tumor immunity. Front Immunol 11: 609948, 2020.