NFκB Targeting in Bone Marrow Mesenchymal Stem Cell-Mediated Support of Age-Linked Hematological Malignancies.

Pranela Rameshwar (rameshwa@njms.rutgers.edu)
Rutgers New Jersey Medical School https://orcid.org/0000-0003-0434-9034
Lauren S. Sherman
Rutgers New Jersey Medical School
Shyam A. Patel
UMass Medical School: University of Massachusetts Medical School
Marianne Castillo
Rutgers New Jersey Medical School
Rachel Unkovic
Rutgers New Jersey Medical School
Marcello Taborga
Rutgers New Jersey Medical School
Marina Gergues
Rutgers New Jersey Medical School
Shaun Patterson
Rutgers New Jersey Medical School
Jean-Pierre Etchegaray
Rutgers University Newark
Mohammed Jaloudi
Rutgers Cancer Institute of New Jersey
Anupama Nehra
Rutgers Cancer Institute of New Jersey
Joshua Kra
Rutgers New Jersey Medical School
Darling P. Rojas
Rutgers School of Dental Medicine Department of Diagnostic Sciences
Victor T. Chang
VA New Jersey Health Care System

Research Article

Keywords: bone marrow, leukemia, myelodysplasia, myeloproliferative disorder mesenchymal stem cell, bortezomib, azacytidine

Posted Date: July 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-672889/v1

Version of Record: A version of this preprint was published at Stem Cell Reviews and Reports on August 19th, 2021. See the published version at https://doi.org/10.1007/s12015-021-10235-6.

Abstract

Mesenchymal stem cells (MSCs) can become dysfunctional in patients with hematological disorders. An unanswered question is whether age-linked disruption of the bone marrow (BM) microenvironment is secondary to hematological dysfunction or vice versa. We therefore studied MSC function in patients with different hematological disorders and found decreased MHC-II except from one sample with acute myeloid leukemia (AML). The patients' MSCs were able to exert veto properties except for AML MSCs. While the expression of MHC-II appeared to be irrelevant to the immune licensing of MSCs, AML MSCs lost their ability to differentiate upon contact and rather, continued to proliferate, forming foci-like structures. We performed a retrospective study that indicated a significant increase in MSCs, based on phenotype, for patients with BM fibrosis. This suggests a role for MSCs in patients transitioning to leukemia. NFκB was important to MSC function and was shown to be a potential target to sensitize leukemic CD34+/CD38- cells to azacitidine. This correlated with their lack of allogeneic stimulation. This study identified NFκB as a potential target for combination therapy to treat leukemia stem cells and showed that understanding MSC biology and immune response could be key in determining how the aging BM might support leukemia. More importantly, we show how MSCs might be involved in transitioning the high risk patient with hematological disorder to AML.

Introduction

Mesenchymal Stem Cells (MSCs), also referred as bone marrow (BM) stromal cells, are found in adult and fetal tissues (1–3). In adults, MSCs are predominantly found in adipose tissue and BM (1, 2). MSCs are distinct from BM stromal cells, which are mostly comprised of hematopoietic supporting fibroblasts, differentiated from MSCs (3–5). In BM, MSCs can be located at different anatomical sites - central sinus, trabeculae, endosteal region and compact bone (5, 6). These locations are also sites of hematopoietic activity in which the function of hematopoietic stem cells (HSCs) are supported with MSCs and their differentiated cells (7). The latter include fibroblasts, adipocytes and osteoblasts (8). Thus, the functional relationship between MSCs and hematopoietic activity are part of the process to maintain BM homeostasis.

MSCs are source of multiple cytokines and then can also respond to cytokines through specific receptors (9–12). As third party cells within an inflammatory milieu such as graft versus host disease (GvHD), MSCs can be licensed as immune suppressor cells to modulate the inflammation (10). At the perivascular region, MSCs can be considered as gate-keeper cells since they can exert plasticity to function as immune suppressor or enhancer cells. In this regard, MSCs can elicit immune stimulation against invading agents. Such response can increase inflammation, which will negatively regulate MSCs as immune suppressor to prevent exacerbated inflammation to avert inflammatory-mediated hematopoietic suppression and premature BM aging (10, 13, 14).

BM MSCs are linked to the pathogenesis of hematological malignancies (15). In light of the immune properties of MSCs and their key role in the hematopoietic niche, it is expected that MSCs might not be able to regulate autoimmune-mediated hematological disorders (15). Indeed, MSCs from patients with idiopathic thrombocytopenia (ITP) exhibit reduced capacity to elicit immune suppression such as enhanced suppressor T-cells to control the autoimmune process of ITP (16). However, the dysfunction in ITP MSCs could be reversed as reported for thalidomide (17). MSCs from aplastic anemia patients, another autoimmune-mediated hematological disorder, also show reduced efficiency to support hematopoiesis (18).

The clinical and experimental evidence indicated that MSCs support the survival of leukemia cells, including drug resistance. Asparaginase, which is used to treat patients with acute lymphocytic leukemia (ALL) to deplete arginine from the leukemic cells, can survive by arginine released from the patients' MSCs (19). Acute myeloid leukemia cells (AML) can survive chemotherapy through the transfer of MSC-derived mitochondria (20). Chronic lymphocytic leukemia (CLL) cells mediate their own survival by releasing microvesicles that differentiate MSCs into cancer associated fibroblasts (21). In addition, PDGF from CLL cells can switch MSCs towards angiogenesis (22). In some cases of known mutation such as myeloproliferative disorder (MPD), the associated MSCs do not seem to harbor the same mutation (23). In contrast, AML and myelodysplastic syndrome (MDS) have chromosomal abnormalities similar to the tumor cells (24, 25). However, others reports show a lack of clonal-associated markers in MDS MSCs with functional competence with respect to hematopoietic support (26). This function is independent of their ability to be immune suppressor veto cells, underscoring their immune dysfunction in hematological disorders (27). MSCs from AML show impaired proliferation, differentiation and hematopoietic support (28). In light the discussed reports on MSCs on hematological disorder, this study focused on the functional immune properties of MSCs to determine how these cells contribute to hematological malignancies. We noted enhanced ability of patients' MSCs to be licensed as immune suppressor cells with an inflammatory milieu and showed that these cells are increased in MF MSCs. Functionally, MSCs appear to require CD74-NFκB axis.

Materials And Methods

Reagents

Dulbecco's modified Eagle's medium (DMEM) with high glucose, RPMI 1640, Phytohemagglutinin (PHA, diluted in tissue culture grade phosphate buffered saline/PBS) and L-glutamine were purchased from Life Technologies Gibco (Grand Island, NY, USA). Fetal calf serum (FCS), paraformaldehyde, tissue culture grade PBS, pH 7.4, bovine serum albumin (BSA) and Ficoll-Hypaque were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bortezomib and azacytidine were obtained from University Hospital Pharmacy, Newark, NJ. RelA Ser529/536 and control peptides were purchased from Novus Biologicals (Centennial, CO). The peptides were dissolved in PBS and used in the assay at 30 μM.

Antibodies

HLA-DR-PE mAb (1/500), CD90-FITC (1/20), CD34-FITC (1/20), CD14-PE, CD45-FITC (1.20), CD29-PE, CD73-PE (1/20), CD44-APC (1/20) CD105-FITC (1/100), fluochrome-isotype IgG and PE-CD34 mAb (1/20) and were purchased from BD PharMingen (San Jose, CA); anti-CD31 mAb (1/100) from Agilent Dako (Santa Clara, CA) and Tri-color human CD45 mAb (1/20) from Caltag ThermoFisher.

Ethical statement

The use of human BM aspirates and peripheral blood (PB) from healthy subjects and patients were approved by the Institutional Review Board of Rutgers University, Newark Campus and VA New Jersey Health Care System, East Orange, New Jersey. All subjects signed the consent forms. Patients' BM aspirates remaining after clinical analyses were used for the research studies.

Patient samples

Aspirates to culture MSCs:

The patient demographics (Table 1**)** comprised the following: Mixed myelodysplastic syndrome myeloproliferative disorders; Polycythemia Vera (PV); Chronic Myeloid Leukemia (CML); Acute Myeloid Leukemia (AML).

BM biopsy slides

Slides (Table 2) from different BM biopsies at VA New Jersey Health Care system for immunohistochemistry. The slides were provided to the study team who were blinded with respect to patients' diagnoses. After labeling and summary of the staining, Dr. Chang provided the codes which were matched to diagnosis and age.

PB/BM aspirates for CD34+/CD38- cell selection

The following donors were selected to isolate CD34+/CD38- cells for drug testing: MF converted to acute leukemia (PB), MDS converted to AML (BM aspirate), CML (PB) and AML (BM aspirates).

Culture of Mesenchymal Stem Cells (MSCs)

MSCs were cultured from BM aspirates of healthy donors and patients with hematological disorders, as described (29). Briefly, unfractionated aspirates were diluted in DMEM with 10% FCS (D10 media) and then added to vacuum gas plasma-treated plates (BD Falcon; Franklin Lakes, NJ). After 3 days, red blood cells and granulocytes were removed by Ficoll-Hypaque density gradient centrifugation and the mononuclear fractions replaced in the plates with fresh D10 media. At weekly intervals, fresh media replaced 50% of the culture media. The adherent cells were serially passage at 80% confluence. At passage 4, the cells were negative for CD34, CD45 and CD14; positive for CD29, CD73, CD90, CD105 and CD44, and differentiated into adipogenic and osteogenic cells (10).

Flow Cytometry

At 80% confluence, MSCs were de-adhered with dissociation solution, washed with 1x PBS (pH 7.4), and then incubated for 30 mins at room temperature with primary antibodies at the dilutions shown above or isotype control. Cells were fixed with 0.2% paraformaldehyde and then analyzed on the FACScan (FACS Caliber; Becton Dickinson, Franklin Lakes, NJ).

Mixed Lymphocyte Response (MLR)

The MLR assay was previously described (10). Briefly, peripheral blood mononuclear cells (PBMCs) (responders) were incubated with MSC stimulator cells in 96-well flat bottom tissue culture plates. The stimulator cells were γ-irradiated at 20 Gy with a Cesium source. After 72 h incubation, each well was pulsed with 1 μCi of [methyl-3H]TdR. After 16 h, the cells were harvested on glass fiber filters, which were analyzed on a scintillation counter for TdR incorporation. Counts per minute (CPM) were converted to disintegration per minute (DPM), which was used to calculate simulation indices (S.I.) = Experimental DPM/PBMC background DPM.

Blast cell isolation

The selection of CD34+/CD38- followed a 2-step isolation protocol. First, CD34+ cells were isolated from the peripheral blood (PB) of patients with leukemia using Dynabeads M-450 (ThermoFisher) as described (30). Briefly, PB mononuclear cells (PBMCs) were isolated by Ficoll Hypaque density gradient centrifugation and 10^7 PBMCs/mL were resuspended in cold Ca^{2+} Mg^{2+}-free PBS containing 2% BSA (isolation buffer). The cell suspension was incubated in the cold for 30 min with 10^8 Dynabeads M-450 CD34. During incubation, the tubes were gentle agitated at 5-min intervals. Cells that bound to the Dynabeads were magnetically selected with Dynal MPC and then washed with isolation buffer. The cells were uncoupled from the beads by incubating at 37^0C for 15 min with DETACHaBEAD CD34 liquid. We verified the isolation efficiency to be ~90%, based on flow cytometry with PE-anti-CD34 and Tri-color-anti-CD45. Non-specific labeling was assessed with non-immune PE-IgG1. Second, CD34+ cells were if they express CD38 by negative selection. This was accomplished by incubating with murine anti-CD38 followed by depletion with Dynabead-linked anti-mouse IgG (ThermoFisher).

Drug treatment

Leukemia blasts (10^3) were added to round bottom 96-well tissue culture plates in 150 μL RPMI 1640 containing 10% FCS. The cells were treated with bortezomib and/or azacytidine, in triplicate, with bortezomib studied at 100 ng/mL and azacytidine at 1 μg/mL. At days 3 and 5, the total number of viable cells were determined by cell count and trypan blue exclusion.

Mitogen Assay

PBMCs from heathy donors were stimulated with 1% PHA in RPMI 1640 containing 10% FCS, as described (10). Healthy or patients' MSCs (10^3/0.2 mL) were added to each well. Control wells contained media instead of MSCs. The cultures were pulsed with 1 µCi/well of [methyl-3H]TdR. Cells were harvested with a PhD cell harvester onto glass-fiber filters. [3H]TdR incorporation was measured in a scintillation counter.

Immunohistochemistry

Antigens from coded paraffin-embedded human BM biopsies were retrieved by overnight incubation at 56^0C. This was followed by dewaxing with xylene, ethanol dehydrationed and rehydration with deionized water. The slides were incubated overnight at 37^0C in the dark within a humidified chamber in which the slides were covered with the primary fluorochrome-tagged antibodies (CD31 or CD105). The antibody concentrations are stated above. Unbound antibodies were washed with 1X PBS and then immediately analyzed on the EVOS FL Auto 2 Imaging System.

Western Blot

Whole cell extracts were obtained from uninduced MSC and induced MSCs. The MSCs were stimulated with IL-1a and TGFβ1 or unstimulated. Cells were trypsinized and washed in PBS (pH 7.4). Following this, 30 µl of 1x Lysis Buffer (Promega, Madison, WI) was added and the cells were subjected to freeze/thaw cycles in a dry ice/ethanol bath. Cell-free whole cell lysates were obtained by centrifugation at 4,000 *g* for 5 min at 4°C. Total protein was determined with a Bio-Rad DC protein assay kit (Hercules, CA). Extracts (200 µg) were analyzed by western blots using 4-20% SDS-PAGE pre-cast gels (Bio-Rad) and the proteins were transferred onto polyvinylidene difluoride membranes (Perkin Elmer Life Sciences, Boston, MA). Membranes were incubated overnight at 4^0C with primary antibodies and then detected the following day by 2-h incubation with HRP-conjugated IgG. All primary and secondary antibodies were used at final dilutions of 1/1000 and 1/2000, respectively. HRP was developed with chemiluminescence detection reagent (Perkin Elmer Life Sciences). The membranes were stripped with Restore Stripping Buffer (Pierce, Rockford, IL) for reprobing with other antibodies.

Adhesion Assay

Adhesion assays were performed to assess contact-dependent interaction between MSCs and cancer cells, using breast cancer cells as a model. MSCs from healthy subjects were cultured in phenol red-free DMEM supplemented with 10% FBS. Next day, MSCs were transfected with scramble siRNA or Rel A siRNA (Dharmacon-Horizon Discovery (Lafayette, CO). The latter was transfected with DharmaFECT (Thermo Scientific) according to the manufacturers recommendations. Parallel studies were incubated with 30 µM RelA Ser929/536 or control peptide. After 24 h, the cells were seeded at $2x10^4$ cells per well in a vacuum gas plasma-treated 96-well plate and then allowed to adhere to the plates overnight. MDA-MB-231 were labeled with CFSE in the dark at room temperature, quenched with phenol-free DMEM and 10^4 cells added in each well containing monolayer MSCs. After 48 h, total fluorescence (pre-aspiration) was assessed on a microplate reader (SoftMax Pro5 SpectraMax 3) at 485nm/535nm. This was followed by washing 3 times with 1x PBS followed by reading for fluorescence (post-aspiration). Percent adhesion was calculated based on the readout pre-aspiration/washing and post-aspiration/washing via the formula: $[RFU_{post\text{-}aspiration} - RFU_{\mu,empty\ well}] \times 100 / [RFU_{pre\text{-}aspiration} - RFU_{\mu,empty\ well}]$ in which RFU= relative fluorescence unit.

Statistical analyses

Statistical analyses were performed with analysis of variance and Tukey-Kramer multiple comparisons test. $p<0.05$ was considered significant.

Results

MSCs from patients with hematological disorders

MSCs from the enrolled patients were expanded in culture to address the experimental questions posed in this manuscript. The morphology of Passage 3 MSCs from patients and healthy donors were similar (Figure 1A-Representative images). Similarly, they exhibited comparable phenotypes for the following cell surface markers: CD105+, CD73+ and CD45- (Figure 1B). The patients' MSCs were multipotent, based on differentiation into osteogenic cells and neurons, as described (31) (Figure 1C).

MHC-II expression on patients' MSCs

MSCs can mediate both anti- and pro-inflammatory responses, depending on the microenvironment (5, 13, 14, 32, 33). Proinflammatory functions include antigen presentation (13). We therefore examined the patients' MSCs for MHC-II/HLA-DR by flow cytometry (Figure 1D). A subset of MSCs from healthy donors express MHC-II (7). MHC-II was not detected on MSCs from PV, MDS and CML BM whereas the two AML samples showed different MHC-II. This section indicated variation among patients' MSCs with respect to MHC-II expression and highlights AML MSCs with MHC-II.

Third party patients' MSCs in immune suppression

MSCs from AML patients show heterogeneity with respect to MHC-II expression whereas those from other patients analyzed in this study exhibit low MHC-II (Figure 1). Unlike macrophages in which IFNg is proportional to MHC-II expression, in MSCs, while low IFNγ induces MHC-II, high levels decrease MHC-II (31). In cases of high IFNg such as an inflammatory milieu caused by GvHD, MSCs can be licensed as veto cells (10, 34). We therefore asked if patients' MSCs can be licensed as immune suppressor cells, similar to healthy MSCs. This was addressed by adding patients' MSCs as third party cells in 2-way MLR.

First, we verified allogeneic difference between the responder and stimulator PBMCs in MLR assay without MSCs (Figure 2A, open bar). As third party cells in the 2-way MLR studies, healthy MSCs decreased the response by 4 folds, consistent with their reported veto property (10, 34). Similar studies with patients'

MSCs varied depending on the source - except for AML MSCs, which enhanced the proliferative response whereas other patients' MSCs significantly ($p<0.05$) resulted in decreased stimulation indices (S.I.) (Figures 2A and 2B).

Patients' MSCs within an inflammatory milieu caused by mitogen

We next asked if patients' MSCs also suppress mitogenic response. This was addressed in PHA-stimulated PBMCs containing MSCs. Control cultures containing PBMCs and healthy MSCs without PHA (media) resulted in >5-fold S.I. (Figure 2C, far left bar). This was expected because MSCs were not added to an inflammatory milieu and the PBMCs responded to the allogeneic difference of healthy MSCs. Similar analyses with MSCs from MDS patient showed 2-fold S.I. which was significantly ($p<0.05$) less than the decreased by healthy MSCs (Figure 2C, left group). The reduced ability of MDS MSCs to elicit an allogeneic response was in line with undetectable MHC-II (Figure 1).

Mitogenic response using PHA alone showed S.I., ~20 (Figure 2C, open bar). After adding healthy MSCs to the PHA-stimulated PBMCs, the S.I. was reduced by ~50% and with MDS MSCs, by ~90% (Figure 2C, right group). Next, we tested AML and CML MSCs in PHA-stimulated PBMCs (Figure 2D). Control included PHA-stimulated cultures without MSCs (media) (open bar). MSCs from healthy individuals and from CML and MDS patients showed significantly ($p<0.05$) reduced S.I. Similarly, MSCs from AML and CML reduced the S.I. (right group). In summary, regardless of MHC-II expression, MSCs from AML, CML and MDS enhanced the immune suppressive licensing ability in the presence of PHA, as compared to healthy MSCs.

MSC distribution in BM biopsies from myelofibrosis (MF) patients

We noted enhanced immune suppressive licensing of MSC from AML, MDS and MPD patients (Figure 2). The question is whether MSC dysfunction is secondary to the respective hematological malignancies. Since patients with myeloproliferative neoplasms (MPNs) in particular MF, can transition to AML (35), we asked if MSCs are increased in MF BM. Particularly, increased MSCs in the BM of MF patients may contribute to enhanced angiogenesis and fibroblasts in MF BM and may contribute to the risk of these patients transitioning to leukemia (36). Indeed, increased angiogenesis in MF patients is in line with the role of MSCs in angiogenic processes – production of angiogenic factors, differentiation into endothelial cells and supporting the architecture of blood vessels as pericytes (37, 38).

We performed immunohistochemistry for CD31 (endothelial cells) and CD105 (MSCs or endothelial cells) with tissues from BM biopsies of patients with fibrosis (MF and other hematological disorder) or non-hematological disorders (Figure 3 and Table 2). There were few areas in which the red fluorescence (CD31) was in contact with green fluorescence (CD105), suggesting pericytes around blood vessels. In all cases including non-hematological disorder, there were single areas of green (CD105) with wide-spread CD31, indicative of increased angiogenesis in MF BM. A summary of the staining is summarized in the lower section (Figure 3), showing increased MSCs in patients with MF and idiopathic myelofibrosis (IMF) and to a lesser extend for slides from non-hematological disorders.

Loss of contact inhibition by AML MSCs

Flow cytometry for MHC-II on MSCs from patients' BM indicated that AML MSCs, as compared to the other sources, showed varied MHC-II (Figure 1D). Regardless of MHC-II, (change this to a comma and delete the period) unlike healthy and other sources of MSCs, AML MSCs failed to exert veto property as third party cells in 2-way MLR (Figure 2A). Yet, they were able to inhibit the proliferation of PBMCs stimulated with the mitogen, PHA (Figure 1D). We therefore focused on AML MSCs due to their ability to support chemoresistance (39).

We asked if the patients' MSCs at passage 5 showed evidence of transformation. All MSCs were seeded at the same time and then observed for time to achieve confluence. We noted rapid confluence by AML MSCs (Figure 4 – top right panel). Unlike AML MSCs, which showed evidence of transformation, MDS MSCs grew slowly with no evidence of transformation (Figure 4 third row). Others studies using a large cohort of AML MSCs observed no evidence of disease mutation but reported on increased clonogenic potential (40). This report is in line with the loss of contact differentiation, expected for healthy MSCs.

Phenotype of AML MSCs

Due to the loss of contact by AML MSCs, we asked if these cells are still phenotypically MSCs. We addressed this question with a panel of markers that are known to be present or absent on MSCs (Figure 5A). The latter included hematopoietic cells, myeloid and lymphoid markers and the former, CD90, CD44, CD73, CD105. In addition, we also evaluated for MHC-II expression. We evaluated AML MSCs for their ability to differentiate into osteogenic cells (Figure 5B). The results indicated phenotypic and functional MSCs, despite the loss of contact inhibition.

CD74-NFκB axis in MSC function

MSCs expanded from AML patients MSCs were less efficient as veto cells, implying their inability to suppress T-cell activation (Figure 2). As compared to the other patients, MSCs from AML expressed MHC-II (Figures 1 and 5A). We therefore focused on the surface glycoprotein CD74 for the following reasons: it serves as the receptor for migration inhibitory factor (MIF) to mediate cancer progression (41); CD74 serves as the invariant chain for MHC-II and could induce intracellular signal transduction via second messengers to promote cell proliferation, noted for AML MSCs (Figure 5A). Thus, CD74 expression on AML MSCs may explain the increased proliferation as well as MHC-II expression. Indeed, similar to healthy subjects, flow cytometry indicated CD74 on AML MSCs (Figure 6A).

CD74 can induce intracellular signaling via NFκB, ERK, and PI(3)K pathways to promote cell proliferation (42). Since NFκB can also maintain MSC multipotency, we asked if inhibiting its activity could affect the function of MSCs (43). We therefore used a model of breast cancer cells due to their ability to interact with MSCs (44). Specifically, we asked if inhibiting NFκB activity would interfere with their ability to interact with MSCs. We inhibited NFκB activity in

r or with Rel A siRNA. Control MSCs were incubated with vehicle or scramble siRNA. After 24 h, MSCs were

incubated with fluorescence labeled MDA-MB-231 breast cancer cells. After 48 h, we assessed cancer cell adhesion to MSCs by fluorescence scanning. We noted significant (p<0.05) decreases when NFκB was inactivated (Figure 6B). In summary, the results support the involvement of CD74-NFκB axis in MSC function.

Enhanced sensitivity by bortezomib and azacytidine on AML blasts

We isolated CD34+/CD38- cells from the blood of four patients with hematologic malignancies (CML, MDS transitioned to AML, AML, MF transitioned to AML). We cultured the cells in the presence of azacytidine and/or bortezomib for 48 h and then accessed cell viability. Each of the four patient cells tested resulted in <5% viable cells as compared to a range of 56-93% after 3- and 5-day exposure with single drug (Table 3). The resistance to single drug occurred despite enhanced cell proliferation. The results indicated that bortezomib seems to prepare the patient's CD34+CD38- cells to azacytidine treatment.

Discussion

This study addressed an early time point that could be important in preventing age-linked hematological disorders transitioning to leukemia. The former includes patients with MF, PV, MDS and other MPD. The licensing ability of healthy MSCs as immune suppressor cells were maintained in MSCs cultured from the BM of patients with CML and MPDs (Figure 2A). In contrast, MSCs from AML patient acted as immune stimulator cells (Figure 2). Although MSCs from one AML patients expressed MHC-II, similar isolated from another AML patients indicated undetectable MHC-II (Figure 1D). Thus, we could not explain the stimulatory response of AML MSCs to MHC-II. Furthermore, healthy MSCs expressed MHC-II but were effective veto cells (10). The observed difference in AML MSCs was noted at confluence where the cells lost contact inhibition (Figure 4). This contrasted healthy and other patients' MSCs that differentiated upon contact (not shown). Indeed, AML MSCs have been shown to be a support of AML growth and also protect them via mitochondrial transfer (19, 20, 45).

Upon contact, AML MSCs began to form foci, suggesting cell transformation, leading to the following questions: Does transformation occur before the onset of AML? If so, do the transformed MSCs initiate the transformation of hematopoietic cells leading to leukemia? Alternatively, does the AML alter the microenvironment by inducing the transformation of the MSCs for their growth? The latter could occur through interaction with genetic material transfer in secretome such as microvesicles as well as mitochondrial transfer. This study therefore initiated several questions for future studies, not only by our group, but others who may have access to patients and archived samples. It should be noted that despite the apparently transformation by the AML MSCs, these cells were similar to healthy stem cells with respect to phenotype and multipotency (Figure 5). Similar to healthy MSCs, one of the methods used to assess multipotency is transdifferentiation into peptidergic neurons. Indeed AML MSCs were able to form neurons, although with reduced efficiency was much less as compared to healthy MSCs, supporting some functional changes that might be important to assess their role in AML support.

Due to the reduced ability of AML MSCs to elicit an allogeneic response compared to healthy MSCs and also MSCs from other patients, we studied the ability of AML MSCs to reduce an inflammatory response, induced by the mitogen, PHA. Interestingly, AML MSCs decreased the mitogen response significantly more than healthy MSCs (Figure 2, right group). The question is why AML MSCs do not cause overt suppression as third party cells in MLR as compared to MSCs from CML and MPD (Figure 1A). These are highly significant observations that would require in-depth studies since this would be relevant for current and future treatments. Also the findings might be relevant in predicting responses when hematopoietic transplantation is done for patients with hematological malignancies.

Since MPN, MDS and MF are significant age-related disorders, they also posed a high risk for transformation into acute leukemias. However, similar transition also occurs in young patients with similar diagnosis. The current treatment for high grade myelodysplastic syndromes are azacytidine and decitabine, with low response rates at approximately 10%. Similar treatment for MF patients with Ruxolitinib does not seem to increase patient survival. Patients with MF have been reported to have increased reticulin fibers, increased angiogenesis and increased fibroblasts. However, earlier reports showed colony forming units-fibroblasts from the BM of MF patients (46). However, it is unlikely that fibroblasts will form colonies, which are established with MSCs. Interestingly, MSCs from patients with JAK2+ MPD supported the neoplastic cells (47). Indeed, analyzing a large cohort of retrospective samples from patients with different hematological malignancies indicated the expected presence of phenotypic MSCs in all patients but more importantly urgently enhanced amounts in patients with MF and in other hematological disorders showing fibrosis (Figure 3). Since these patients are at high risk to transition into leukemias, this finding was highly significant in the quest to prevent leukemia from patients with disorders such as MDS and MF.

The immunohistochemistry findings led us to seek potential mechanisms for MSCs. We could not find any difference in CD74, which could serve as an invariant chain of MHC-II (41) (Figure 6A). Since CD74 could also activate intracellular signaling, partly through NFκB activation (42), we asked if the latter could be involved in MSC function. Using a model of breast cancer-MSC interaction (44, 48), we showed that inhibition of RelA in MSCs significantly decreased the interaction (Figure 6B), supporting a role for NFκB in MSC function. The effect of NFκB in MSC function was in line with its reported role in maintaining cancer stem cells (49). We identified NFκB, with bortezomib treatment, sensitized the otherwise resistant leukemia stem cells to azacytidine (Table 3). Since one of the patients was transitioning from MDS to AML with ~20% blast, the data suggested that this combination could be a treatment for patients at risk for transition into acute leukemia.

In summary, the combined treatment shown in Table 3 strongly support a large clinical trial. The studies also indicate that MSC function might hold the key to future therapies for hematological disorders and their function might be predictor of responses to varied treatments. Several hematological disorders are immune-mediated. Due to the dual properties of MSCs as immune enhancers and suppressors, it is important to examine these cells at the molecular level. A key finding is the MHC-II invariant chain CD74 that persisted on AML MSCs. This may prevent tumor antigen loading and presentation to T-cells, allowing for immune subversion. These findings will need to be expanded to MSCs derived from other patients because in addition to what is shown in Table 3 for combined treatment, the available anti-CD74, milatuzumab, merits further investigation for combined treatment. The findings in this study might begin to shed a different perspective on the reason for the concentration of leukemic cells in gum where MSCs can be present and with similar function as those in the BM

(50, 51). Additionally, the studies provide insights on how the aging BM microenvironment, particularly MSCs could be poised to support the growth of leukemia cells.

Declarations

Funding: This work was supported by F.M. Kirby Foundation

Conflicts of interest/Competing interests: None

Ethics approval: The use of human subjects received institutional approval (see Materials)

Consent to participate: Patients who donated bone marrow aspirates signed the informed consent form.

Consent for publication: The consent form stated that the data will be published with the subjects deidentified.

Availability of data and material: Material and the raw data will be made available.

Code availability: Not applicable

Authors' contributions:

Conceptualization: Pranela Rameshwar; Victor T. Chang; Lauren Sherman

Methodology: Lauren Sherman, Pranela Rameshwar, Jean-Pierre Etchegaray, Marina Gergues, Rachel Unkovic, Marianne Castillo, Marcello Taborga

Formal analysis and investigation: Rachel Unkovic, Shaun Patterson, Marcello Taborga, Marianne Castillo, Lauren S. Sherman, Pranela Rameshwar, Victor T. Chang

Writing - original draft preparation: Lauren S. Sherman, Marianne Castillo, Shyam A. Patel, Victor T. Chang, Rachel Unkovic, Pranela Rameshwar

Writing - review and editing: Shyam A. Patel, Victor T. Chang, Pranela Rameshwar, Lauren S. Sherman, Darling P. Rojas, Mohammed Jaloudi, Anupama Nehra, Joshua Kra

Funding acquisition: Pranela Rameshwar;

Supervision: Pranela Rameshwar, Victor T. Chang

References

1. Campagnoli C, Roberts IAG, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood. 2001;98:2396-2402.
2. Castillo M, Liu K, Bonilla L, Rameshwar P. The immune properties of mesenchymal stem cells. Int J Biomed Sci. 2007;3:76-80.
3. Caplan AI, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. Trends Mol Med. 2001;7:259-264.
4. Liu M, Miller CL, Eaves CJ. Human long-term culture initiating cell assay. Methods Mol Biol. 2013;946:241-256.
5. Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells. 2001;19:180-192.
6. Anastasio A, Gergues M, Lebhar MS, Rameshwar P, Fernandez-Moure J. Isolation and characterization of mesenchymal stem cells in orthopaedics and the emergence of compact bone mesenchymal stem cells as a promising surgical adjunct. World J Stem Cells. 2020;12:1341-1353.
7. Ghebes CA, Morhayim J, Kleijer M, Koroglu M, Erkeland SJ, Hoogenboezem R, Bindels E, van Alphen FPJ, van den Biggelaar M, Nolte MA, van der Eerden BCJ, Braakman E, Voermans C, van de Peppel J. Extracellular Vesicles Derived From Adult and Fetal Bone Marrow Mesenchymal Stromal Cells Differentially Promote ex vivo Expansion of Hematopoietic Stem and Progenitor Cells. Frontiers Bioeng Biotechnol. 2021;9.
8. Yurova KA, Khaziakhmatova OG, Melashchenko ES, Malashchenko VV, Shunkin EO, Shupletsova VV, Ivanov PA, Khlusov IA, Litvinova LS. Cellular and Molecular Basis of Osteoblastic and Vascular Niches in the Processes of Hematopoiesis and Bone Remodeling (A Short Review of Modern Views). Curr Pharm Des. 2019;25:663-669.
9. Patel SA, Sherman L, Munoz J, Rameshwar P. Immunological properties of mesenchymal stem cells and clinical implications. Arch Immunol Ther Exp. 2008;56:1-8.
10. Potian JA, Aviv H, Ponzio NM, Harrison JS, Rameshwar P. Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. J Immunol. 2003;171:3426-3434.
11. Stagg J, Galipeau J. Mechanisms of immune modulation by mesenchymal stromal cells and clinical translation. Curr Mol Med. 2013;13:856-867.
12. Sasse S, Skorska A, Lux CA, Steinhoff G, David R, Gaebel R. Angiogenic Potential of Bone Marrow Derived CD133(+) and CD271(+) Intramyocardial Stem Cell Trans- Plantation Post MI. Cells. 2019;9.
13. Chan JL, Tang KC, Patel AP, Bonilla LM, Pierobon N, Ponzio NM, Rameshwar P. Antigen-presenting property of mesenchymal stem cells occurs during a narrow window at low levels of interferon-gamma. Blood. 2006;107:4817-4824.

14. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol. 2014;15:1009-1016.
15. Fattizzo B, Giannotta JA, Barcellini W. Mesenchymal Stem Cells in Aplastic Anemia and Myelodysplastic Syndromes: The "Seed and Soil" Crosstalk. Int J Mol Sci. 2020;21.
16. Li H, Guan Y, Sun B, Dou X, Liu X, Xue F, Fu R, Zhang L, Yang R. Role of bone marrow-derived mesenchymal stem cell defects in CD8(+) CD28(-) suppressor T-lymphocyte induction in patients with immune thrombocytopenia and associated mechanisms. Br J Haematol. 2020;191:852-862.
17. Ma J, Ning YN, Xu M, Hou Y, Wang N, Hou XY, Yu YY, Li H, He WD, Shao LL, Zhou H, Min YN, Liu XG, Shi Y, Qin P, Guo CS, Hou M, Peng J. Thalidomide corrects impaired mesenchymal stem cell function in inducing tolerogenic DCs in patients with immune thrombocytopenia. Blood. 2013;122:2074-2082.
18. Hamzic E, Whiting K, Gordon Smith E, Pettengell R. Characterization of bone marrow mesenchymal stromal cells in aplastic anaemia. Br J Haematol. 2015;169:804-813.
19. Iwamoto S, Mihara K, Downing JR, Pui CH, Campana D. Mesenchymal cells regulate the response of acute lymphoblastic leukemia cells to asparaginase. J Clin Invest. 2007;117:1049-1057.
20. Moschoi R, Imbert V, Nebout M, Chiche J, Mary D, Prebet T, Saland E, Castellano R, Pouyet L, Collette Y, Vey N, Chabannon C, Recher C, Sarry JE, Alcor D, Peyron JF, Griessinger E. Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. Blood. 2016;128:253-264.
21. Paggetti J, Haderk F, Seiffert M, Janji B, Distler U, Ammerlaan W, Kim YJ, Adam J, Lichter P, Solary E, Berchem G, Moussay E. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. Blood. 2015;126:1106-1117.
22. Ding W, Knox TR, Tschumper RC, Wu W, Schwager SM, Boysen JC, Jelinek DF, Kay NE. Platelet-derived growth factor (PDGF)-PDGF receptor interaction activates bone marrow-derived mesenchymal stromal cells derived from chronic lymphocytic leukemia: implications for an angiogenic switch. Blood. 2010;116:2984-2993.
23. Mercier F, Monczak Y, François M, Prchal J, Galipeau J. Bone marrow mesenchymal stromal cells of patients with myeloproliferative disorders do not carry the JAK2-V617F mutation. Exp Hematol. 2009;37:416-420.
24. Blau O, Hofmann WK, Baldus CD, Thiel G, Serbent V, Schümann E, Thiel E, Blau IW. Chromosomal aberrations in bone marrow mesenchymal stroma cells from patients with myelodysplastic syndrome and acute myeloblastic leukemia. Exp Hematol. 2007;35:221-229.
25. Alvi S, Shaher A, Shetty V, Henderson B, Dangerfield B, Zorat F, Joshi L, Anthwal S, Lisak L, Little L, Gezer S, Mundle S, Reddy PL, Allampallam K, Huang X, Galili N, Borok RZ, Raza A. Successful establishment of long-term bone marrow cultures in 103 patients with myelodysplastic syndromes. Leuk Res. 2001;25:941-954.
26. Soenen-Cornu V, Tourino C, Bonnet ML, Guillier M, Flamant S, Kotb R, Bernheim A, Bourhis JH, Preudhomme C, Fenaux P, Turhan AG. Mesenchymal cells generated from patients with myelodysplastic syndromes are devoid of chromosomal clonal markers and support short- and long-term hematopoiesis in vitro. Oncogene. 2005;24:2441-2448.
27. Han Q, Sun Z, Liu L, Chen B, Cao Y, Li K, Zhao RC. Impairment in immuno-modulatory function of Flk1(+)CD31(-)CD34(-) MSCs from MDS-RA patients. Leuk Res. 2007;31:1469-1478.
28. Zhao ZG, Liang Y, Li K, Li WM, Li QB, Chen ZC, Zou P. Phenotypic and functional comparison of mesenchymal stem cells derived from the bone marrow of normal adults and patients with hematologic malignant diseases. Stem Cells Dev. 2007;16:637-648.
29. Bliss SA, Sinha G, Sandiford OA, Williams LM, Engelberth DJ, Guiro K, Isenalumhe LL, Greco SJ, Ayer S, Bryan M, Kumar R, Ponzio NM, Rameshwar P. Mesenchymal Stem Cell-Derived Exosomes Stimulate Cycling Quiescence and Early Breast Cancer Dormancy in Bone Marrow. Cancer Res. 2016;76:5832-5844.
30. Rameshwar P, Zhu G, Donnelly RJ, Qian J, Ge H, Goldstein KR, Denny TN, Gascón P. The dynamics of bone marrow stromal cells in the proliferation of multipotent hematopoietic progenitors by substance P: an understanding of the effects of a neurotransmitter on the differentiating hematopoietic stem cell. J Neuroimmunol. 2001;121:22-31.
31. Castillo MD, Trzaska KA, Greco SJ, Ponzio NM, Rameshwar P. Immunostimulatory effects of mesenchymal stem cell-derived neurons: implications for stem cell therapy in allogeneic transplantations. Clin Transl Sci. 2008;1:27-34.
32. Desai MB, Gavrilova T, Liu J, Patel SA, Kartan S, Greco SJ, Capitle E, Rameshwar P. Pollen-induced antigen presentation by mesenchymal stem cells and T cells from allergic rhinitis. Clin Transl Immunol. 2013;2:e7.
33. Németh K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009;15:42-49.
34. Galipeau J, Sensébé L. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. Cell Stem Cell. 2018;22:824-833.
35. Bose P, Verstovsek S. The evolution and clinical relevance of prognostic classification systems in myelofibrosis. Cancer. 2016;122:681-692.
36. Lekovic D, Gotic M, Skoda R, Beleslin-Cokic B, Milic N, Mitrovic-Ajtic O, Nienhold R, Sefer D, Suboticki T, Buac M, Markovic D, Diklic M, Cokic VP. Bone marrow microvessel density and plasma angiogenic factors in myeloproliferative neoplasms: clinicopathological and molecular correlations. Ann Hematol. 2017;96:393-404.
37. Konno M, Hamazaki TS, Fukuda S, Tokuhara M, Uchiyama H, Okazawa H, Okochi H, Asashima M. Efficiently differentiating vascular endothelial cells from adipose tissue-derived mesenchymal stem cells in serum-free culture. Biochem Biophys Res Commun. 2010;400:461-465.
38. Hashitani H, Mitsui R. Role of Pericytes in the Initiation and Propagation of Spontaneous Activity in the Microvasculature. Adv Exp Med Biol. 2019;1124:229-256.

39. Yuan B, El Dana F, Ly S, Yan Y, Ruvolo V, Shpall EJ, Konopleva M, Andreeff M, Battula VL. Bone marrow stromal cells induce an ALDH+ stem cell-like phenotype and enhance therapy resistance in AML through a TGF-β-p38-ALDH2 pathway. PLoS One. 2020;15:e0242809.
40. 40. Diaz de la Guardia R, Lopez-Millan B, Lavoie JR, Bueno C, Castaño J, Gómez-Casares M, Vives S, Palomo L, Juan M, Delgado J, Blanco ML, Nomdedeu J, Chaparro A, Fuster JL, Anguita E, Rosu-Myles M, Menéndez P. Detailed Characterization of Mesenchymal Stem/Stromal Cells from a Large Cohort of AML Patients Demonstrates a Definitive Link to Treatment Outcomes. Stem Cell Reports. 2017;8:1573-1586.
41. Schröder B. The multifaceted roles of the invariant chain CD74 - More than just a chaperone. Biochimica et Biophysica Acta - Mol Cell Res. 2016;1863:1269-1281.
42. Starlets D, Gore Y, Binsky I, Haran M, Harpaz N, Shvidel L, Becker-Herman S, Berrebi A, Shachar I. Cell-surface CD74 initiates a signaling cascade leading to cell proliferation and survival. Blood. 2006;107:4807-4816.
43. Chang J, Liu F, Lee M, Wu B, Ting K, Zara JN, Soo C, Al Hezaimi K, Zou W, Chen X, Mooney DJ, Wang C-Y. NF-κB inhibits osteogenic differentiation of mesenchymal stem cells by promoting β-catenin degradation. Proc Natl Acad Sci. 2013;110:9469-9474.
44. 44. Patel SA, Dave MA, Bliss SA, Giec-Ujda AB, Bryan M, Pliner LF, Rameshwar P. T(reg)/Th17 polarization by distinct subsets of breast cancer cells is dictated by the interaction with mesenchymal stem cells. J Cancer Stem Cell Res. 2014;2014.
45. 45. Corradi G, Baldazzi C, Očadlíková D, Marconi G, Parisi S, Testoni N, Finelli C, Cavo M, Curti A, Ciciarello M. Mesenchymal stromal cells from myelodysplastic and acute myeloid leukemia patients display in vitro reduced proliferative potential and similar capacity to support leukemia cell survival. Stem Cell Res Ther. 2018;9:271.
46. Castro-Malaspina H, Gay RE, Jhanwar SC, Hamilton JA, Chiarieri DR, Meyers PA, Gay S, Moore MA. Characteristics of bone marrow fibroblast colony-forming cells (CFU-F) and their progeny in patients with myeloproliferative disorders. Blood. 1982;59:1046-1054.
47. Ramos TL, Sánchez-Abarca LI, Rosón-Burgo B, Redondo A, Rico A, Preciado S, Ortega R, Rodríguez C, Muntión S, Hernández-Hernández Á, De Las Rivas J, González M, González Porras JR, Del Cañizo C, Sánchez-Guijo F. Mesenchymal stromal cells (MSC) from JAK2+ myeloproliferative neoplasms differ from normal MSC and contribute to the maintenance of neoplastic hematopoiesis. PLoS One. 2017;12:e0182470.
48. Greco SJ, Patel SA, Bryan M, Pliner LF, Banerjee D, Rameshwar P. AMD3100-mediated production of interleukin-1 from mesenchymal stem cells is key to chemosensitivity of breast cancer cells. Am J Cancer Res. 2011;1:701-715.
49. Yamamoto M, Taguchi Y, Ito-Kureha T, Semba K, Yamaguchi N, Inoue J. NF-κB non-cell-autonomously regulates cancer stem cell populations in the basal-like breast cancer subtype. Nat Commun. 2013;4:2299.
50. Pagella P, de Vargas Roditi L, Stadlinger B, Moor AE, Mitsiadis TA. A single-cell atlas of human teeth. iScience. 2021;24:102405.
51. Angst PDM, Maier J, Dos Santos Nogueira R, Manso IS, Tedesco TK. Oral health status of patients with leukemia: a systematic review with meta-analysis. Arch Oral Biol. 2020;120:104948.

Tables

Table 1. Patient demographics with BM aspirates

Patient	DIAGNOSIS	AGE (YRS)	SEX	TREATMENT
1	Acute Myeloid Leukemia (AML)	83	M	NONE
2	Acute Myeloid Leukemia (AML	83	M	
3	Chronic Myeloid Leukemia (CML)	75	M	NONE
4	Myelodysplastic Syndrome with Myeloproliferative Disorder (MDS/ MPD)	65	M	Thalidomide Erythropoietin Anagrelide
5	Polycythemia Vera (PV)	75	M	NONE
6	Control (No Hematological Disorder)	75	M	NONE

Table 2. Demographics of BM biopsy slides

Diagnosis	Age Range (Yrs)	Total
MF	75-88	13
PV with Fibrosis	64-77	4
MDS with Fibrosis	67-71	2
MPD with Fibrosis	60-90	5
Mild/Moderate Fibrosis	66-82	3
CML with Fibrosis	62-77	2
ET with Fibrosis	75	1
MPD	62-81	6
MDS	72	1
CMML	43-66	2
ET	54-59	2
CLL	81	1
CML	59-77	3
MDS/MPD	63-84	2
MDS to AML	81	1
AML	55-82	4
Non-Hematological	54-85	10
Total		**62**

AML: Acute Myeloid Leukemia; CLL: Chronic Lymphocytic Leukemia; CML: Chronic Myeloid Leukemia; CMML: Chronic Myelomoncytic Leukemia; ET: Essential Thrombocytemia; MF: Myelofibrosis; MDS: Myelodysplastic Syndrome; MPD: Myeloproliferative disorder; PV: Polycythemia Vera

Table 3. Effects of Bortezomib and/or Azacytidine on blast cells

Diagnosis	Sample	Bortezomib		Azacytidine		Bortezomib+ Azacytidine	
		% Viability					
		D3	D5	D3	D5	D3	D5
CML	Blood	79	80	84	93	5	3
AML	BM Aspirates	70	75	63	65	2	2
MDS-AML	BM Aspirates	78	83	55	42	2	1
MF-Acute Leukemia	Blood	72	80	56	52	3	2

Viable CD34+/CD38- cells following 48 h exposure to Bortezomib and/or azacytidine.

Figures

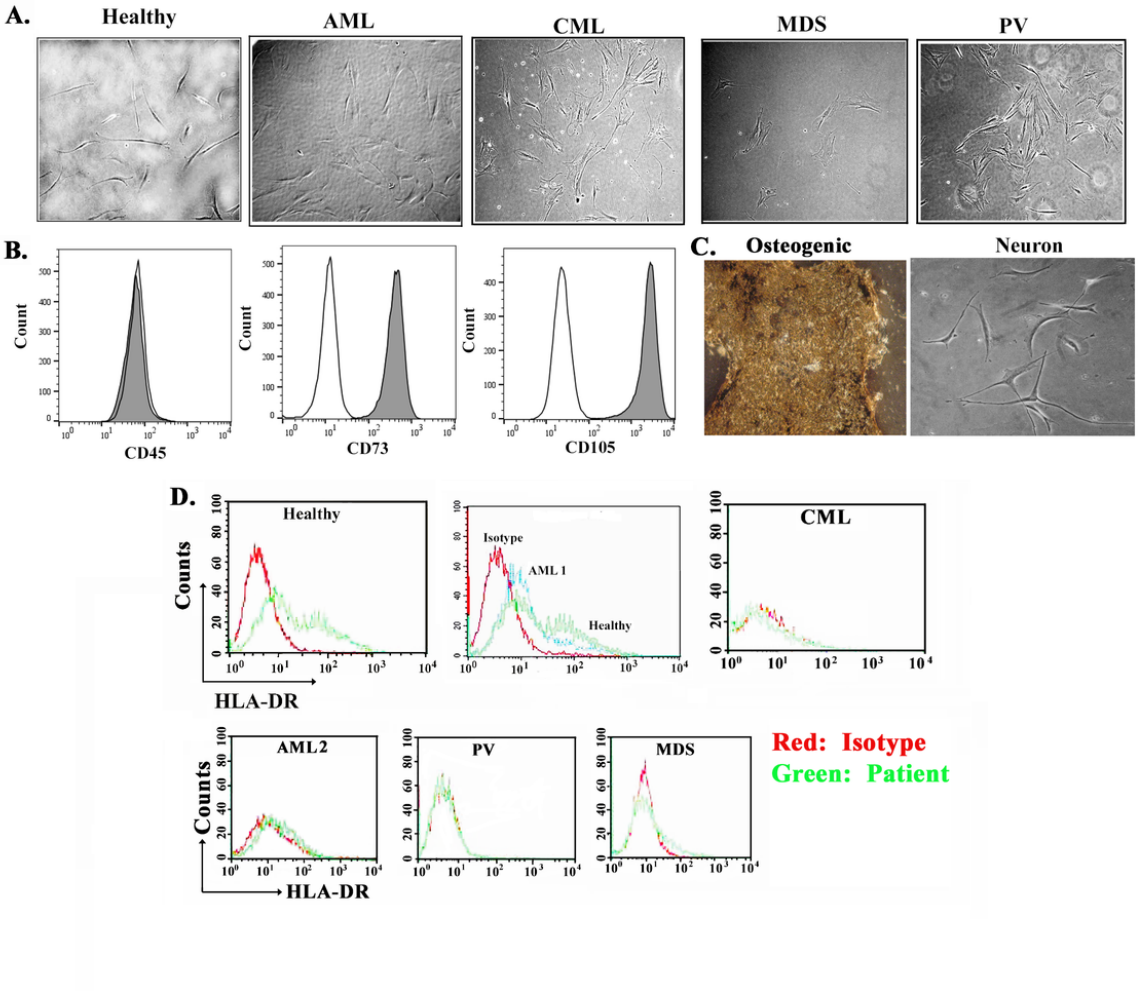


Figure 1

MHC-II on patients' MSCs. A) Representative morphology of passage 3 MSCs from healthy subjects and patients. B) Representative histogram for CD45, CD73 and CD105 from flow cytometry of MSCs expanded from healthy subjects and patients. C) Representative osteogenic and neurons derived from healthy and patients' MSCs. D) Flow cytometry for HLA-DR on MSCs from patients without hematological malignancies (Healthy) and patients' MSCs (AML, PV, MDS, CML). Each analysis included an isotype control.

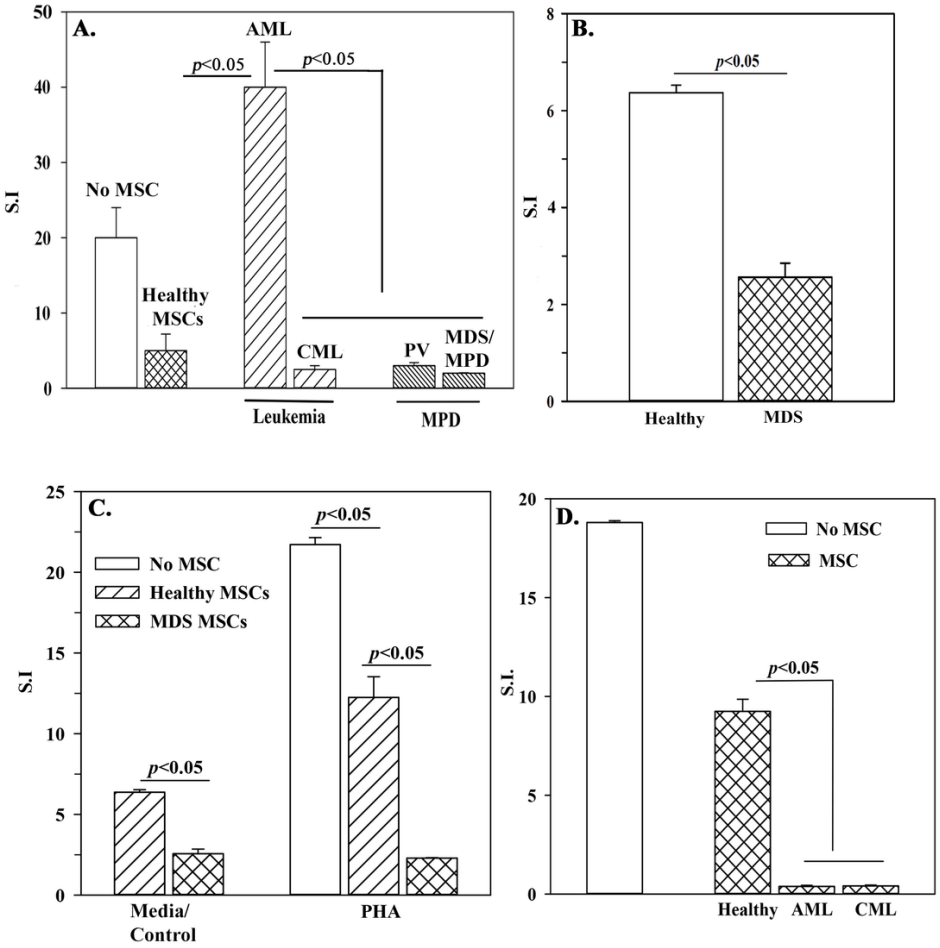
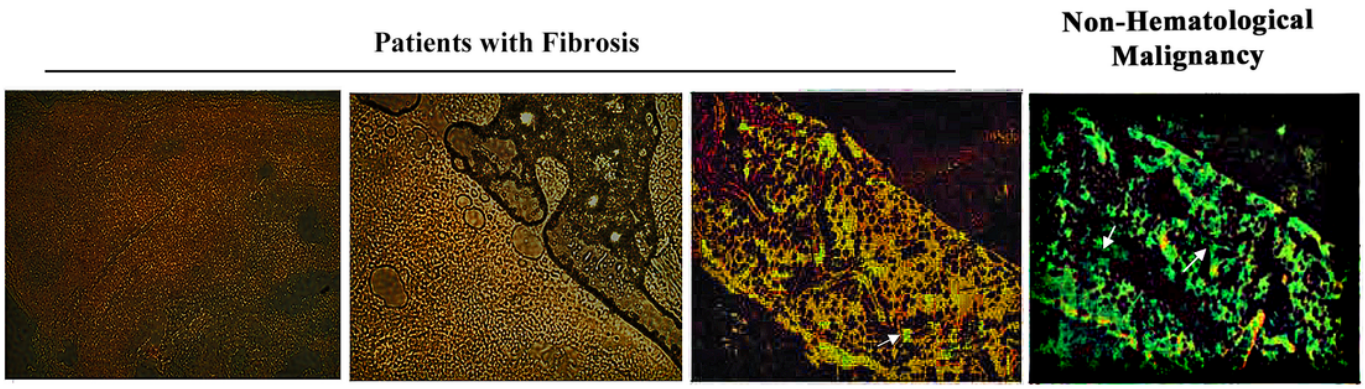


Figure 2

Immune response by patients' MSCs. A) Two-way MLR was performed with or without MSCs as third-party cells from healthy controls and from patients (AML, CML, MPD). Each experimental point is the mean of four different experiments. The data are expressed as stimulation indices (S.I.), ±SD. B) One way MLR was performed with irradiated stimulator MSCs from healthy subjects or MDS and the results presented as for

$A', mean \pm SD, n = 4. C \Big) PBMCswerestiμlatedwithPHA \in thepresence$ or $|e|nceofMSCsomMDS$ or $heathydon$ or $s. Controlstudies \in clude$

C' was repeated and MSCs from patients (AML and CML) or healthy control was added to the PHA-stimulated cultures and the results presented as for `C', mean S.I.±SD, n=4.



Diagnosis	Total Number	CD31+	CD105+	Co-localized CD31/CD105
Non-Hematology Disorder	10	+	+	Punctate
IMF	5	+++	+++	Diffuse
MF	8	+++	+++	Diffuse
Moderate Reticulin Fibrosis	10	+++	+++	Diffuse

Figure 3

Immunohistochemistry for MSC in BM biopsies of patients with fibrosis. Slides (Table 2) with sections from paraffin-embedded tissues provided and blindly labeled with anti-CD105-FITC and anti-CD31-PE. Top images show representative labeling and the lower panel, a summary of the analyzed tissues. Arrows show CD105+/CD31- cells.

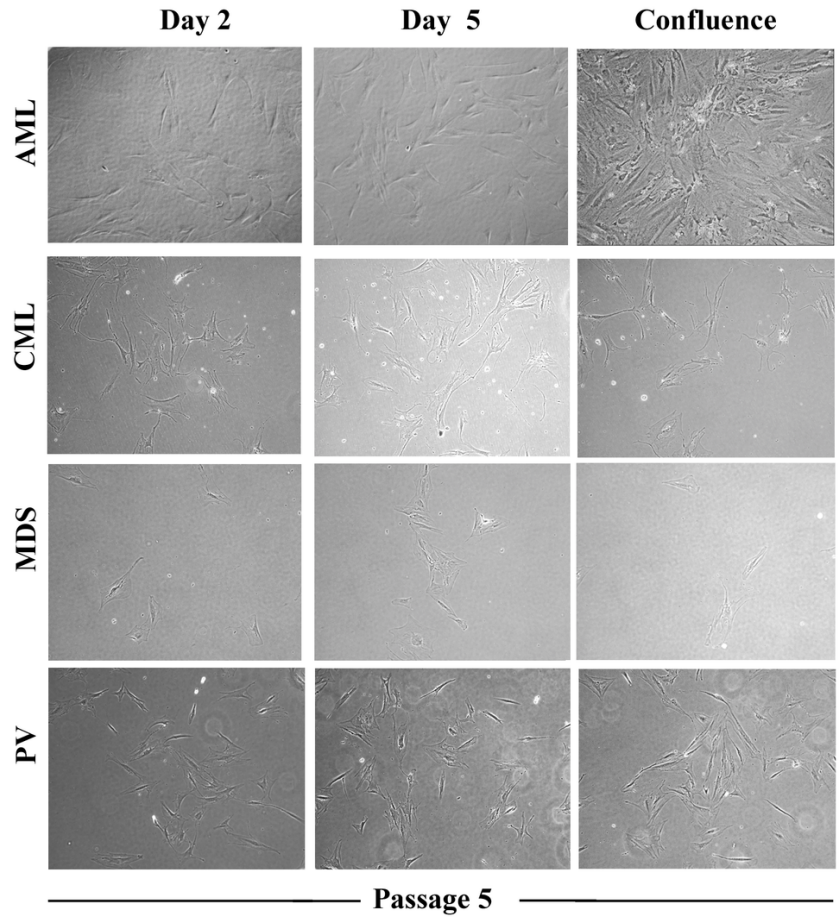


Figure 4

Morphology of Patient's MSCs. Phase contrast images (200x) of MSCs from patients at passage 5. The images are shown at Days 2 and 5, post-seeding and at confluence.

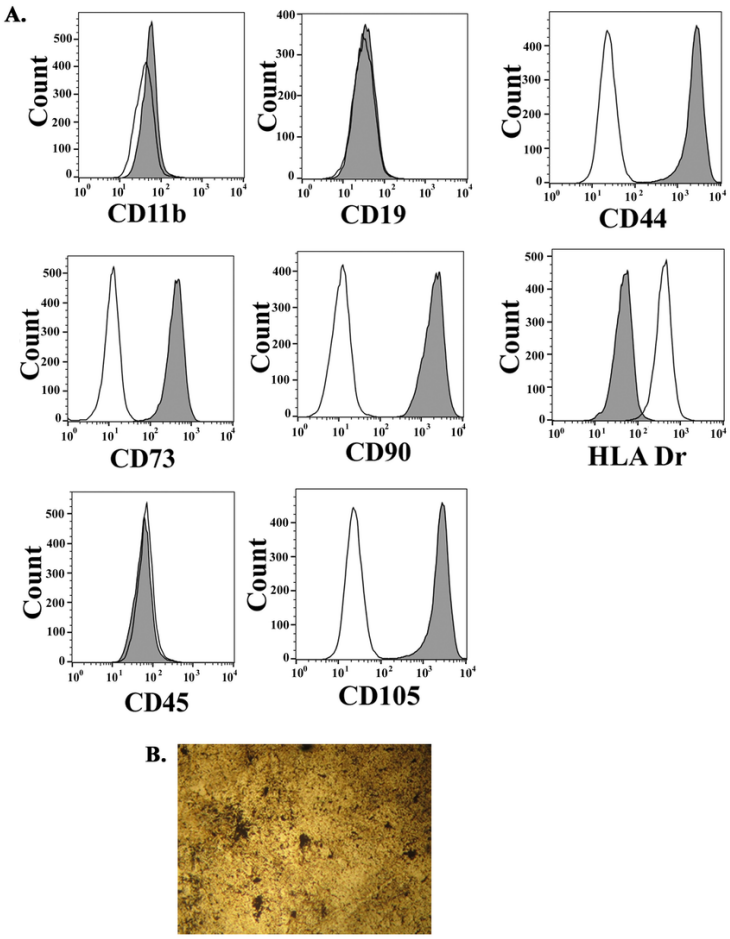


Figure 5

Characterization of AML MSCs. A) Expanded MSCs from AML aspirates were analyzed by flow cytometry for myeloid (CD11b), lymphoid (CD19), hematopoietic cell (CD45), HLA-DR and know markers of MSCs. The results represent three different analyses. B) MSCs at 80% confluence were studied for osteogenic differentiation.

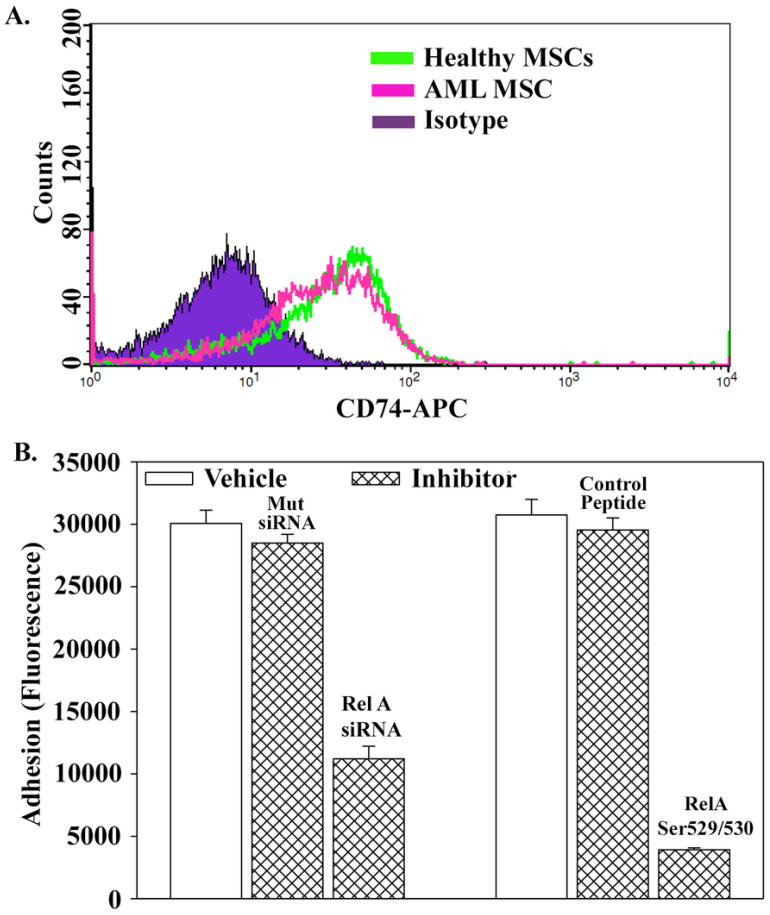


Figure 6

NFκB signaling in MSCs. A) Representative histogram for flow cytometry for CD74 on MSCs from healthy donors and AML. B) MSCs were knockdown for RelA or pretreated with the inhibitor, RelA Ser529/530 and then evaluated for adhesion to MDA-MB-231 breast cancer cells. The results are the mean±SD, n=3. Each experiment was performed in triplicate and each with MSCs from a different donor.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.pptx