J. Cell Commun. Signal. (2018) 12:441–450
DOI 10.1007/s12079-017-0412-8

RESEARCH ARTICLE

K562 chronic myeloid leukemia cells modify osteogenic differentiation and gene expression of bone marrow stromal cells

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Received: 26 June 2017 / Accepted: 20 September 2017 / Published online: 30 September 2017

Abstract Bone marrow (BM) microenvironment plays an important role in normal and malignant hematopoiesis. As a consequence of interaction with the leukemic cells, the stromal cells of the bone marrow become deregulated in their normal function and gene expression. In our study, we found that mesenchymal stem cells (MSC) from BM of chronic myeloid leukemia (CML) patients have defective osteogenic differentiation and on interaction with K562 CML cells, the normal MSC showed reduced osteogenic differentiation. On interaction with K562 cells or its secreted factors, MSC acquired phenotypic abnormalities and secreted high levels of IL6 through NFκB activation. The MSC derived secreted factors provided a survival advantage to CML cells from imatinib induced apoptosis. Thus, a therapy targeting stromal cells in addition to leukemia cells might be more effective in eliminating CML cells.

Keywords Bone marrow stroma · Osteoblasts · Therapy resistance · IL6, NFκB signaling · Paracrine factors · Cell-cell communication

Abbreviations

ADIPOQ	Adiponectin
ALP	Alkaline phosphatase
AML	Acute myeloid leukemia
AZR	Alizarin red
BM	Bone marrow
BSP	Bone sialoprotein
CAT	Catalase
CML	Chronic myeloid leukemia
HSC	Hematopoietic stem cells
IL6	Interleukin 6
IM	Imatinib mesylate
MnSOD	Manganese superoxide dismutase
MSC	Mesenchymal stem cells
NFκB	Nuclear factor kappa B
OCN	Osteocalcin
ORO	Oil red O
PlGF	Placental growth factor
TNFα	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

Introduction

BM microenvironment stromal cells have been extensively studied for their role in normal hematopoiesis and modifications in the stromal cells were reported to disrupt HSC maintenance and differentiation (Morrison and Scadden 2014). BM stromal cells support and regulate normal and leukemic stem cells by providing signaling cues for the maintenance, proliferation, survival, migration, homing and differentiation (Levesque et al. 2010; Lo Celso and Scadden 2011). Leukemic cells were proposed to hijack and manipulate the normal BM microenvironment and render it more supportive for leukemic cells and less suitable

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12079-017-0412-8) contains supplementary material, which is available to authorized users.

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for normal hematopoiesis (Krause and Scadden 2015) and play an important role in chemoresistance development (Kumar et al. 2017). BM stroma was implicated in leukemia initiation where dysfunctional osteoprogenitor cells or osteoblasts in the mouse BM resulted in deregulation of normal hematopoiesis, leading to myelodysplastic syndrome (MDS) which further gave rise to acute myeloid leukemia (AML) (Kode et al. 2016; Raaijmakers et al. 2010). Mutations have also been reported in MSC isolated from patients with hematologic malignancies. MSC from MDS and AML were reported to carry cytogenetic abnormalities which were distinct from those seen in leukemic cells (Blau et al. 2011). However, MSC derived from CML patients did not have BCR-ABL fusion gene present in the hematopoietic cells (Wohrer et al. 2007). MSC isolated form hematological malignancies also carry functional differences compared to MSC derived from healthy individuals (Campioni et al. 2009; Huang et al. 2015). In addition, stromal cells are a major source of cytokines and growth factors. High levels of pro-survival cytokines were identified in the bone marrow aspirates of cancer patients (Civini et al. 2013; Semesiuk et al. 2013) which might support the cancer cell proliferation and cancer chemoresistance.

In our study, we found that MSC isolated form bone marrow of CML patients have defective osteogenic differentiation, altered cell surface marker expression and modified gene expression. Interaction with CML cells also resulted in high IL6 expression in MSC and paracrine factors from MSC in turn chemoprotected CML cells from imatinib mesylate (IM) induced apoptosis.

Materials and methods

Isolation of bone marrow derived MSC

Bone marrow aspirates were obtained from patients referred for bone marrow biopsy following protocols approved by local ethical committee and after informed consent from the patients included in the study. Bone marrow samples of BCR-ABL positive CML patients were obtained at chronic phase during diagnosis (7 samples) or patients at remission stage but on imatinib therapy for 2 months (4 samples) from both male and female patients with a median age of 40 years. Bone marrow from patients who were confirmed to have no hematologic malignancies after bone marrow analysis were considered as control samples (Control-MSC). MSC were isolated from the bone marrow samples after red cell lysis and plated in growth media as described previously (Sonowal et al. 2013). The non-adherent cells were removed and the adherent spindle shaped cells were expanded further and characterized.

Differentiation of MSC

MSC were differentiated into adipocytes, osteoblasts or chondrocytes as described previously (Mawrie et al. 2016; Somaiah et al. 2015). Briefly, the cells were cultured in media supplemented with dexamethasone, isobutyl methyl xanthine, indomethacin and insulin to induce adipogenic differentiation and dexamethasone, β-glycerolphosphate, and ascorbic acid (Sigma) to induce osteogenic differentiation for 14 days. Adipogenic differentiation was determined by Oil Red O staining or gene expression analysis of adipo specific gene *ADIPONECTIN* (*ADIPOQ*) and osteogenic differentiation was detected by alizarin Red staining (Sigma) or expression levels of osteogenic genes *OSTEOCALCIN* (*OCN*) or Bone Sialoprotein (*BSP*). Osteogenic differentiation was also determined by histochemical staining for alkaline phosphatase (Sigma). Osteogenic differentiation was quantified by eluting Alizarin red with cetylpyridinium chloride and absorbance measurement at 562 nm. Chondrogenic differentiation was performed by generating micromass cultures in a 12-well plate according to the manufacturer's instructions (Thermofisher scientific). Chondrocyte differentiation was analyzed by Safranin O staining after 21 days of differentiation.

Colony forming unit (CFU-F) analysis

One hundred cells per well were seeded in a 6-well plate and cultured in growth media containing 20% FBS for 14 days. Colonies larger than 50 cells were counted.

Senescence assay

Cells were seeded in a 12 well plate and allowed to grow until 80% confluent. The cells were stained for β-galactosidase activity using histochemical staining kit according to the manufacturer's instructions (Sigma). The stained cells were visualized under microscope and documented.

Flow cytometry

MSC were trypsinized and stained with anti-human fluorescent conjugated antibodies (BD Biosciences) against the indicated cell surface receptors. The cells were incubated at 4°C for 30 min. The cells were washed and analyzed with FACS calibur (BD Biosciences). Propidium iodide was used for live/dead discrimination.

MSC co-culture with CML K562 cells

For co-culture experiments, MSC were seeded and allowed to attach for 24 h. K562 cells (National Centre for Cell Sciences, Pune, India) were added to the MSC and allowed to grow for indicated time periods. K562 cells were removed

from the co-cultured MSC by quick trypsinization for 30 s. For flow cytometric and gene expression analysis, MSC were further trypsinized and used (Suppl. File 1)

Collection of conditioned media

To collect conditioned media from MSC, MSC were seeded and grown to 90% confluence. Fresh complete growth media (RPMI) was added to the cells and conditioned media containing the secreted factors was collected after 3 h and used immediately or frozen at −80°C until required.

To collect conditioned media from K562 cells, the cells were resuspended in fresh growth media (RPMI) at a density of 8×10^5 cells/ml. The cells were incubated for 3 h at 37°C, the cells were separated from the conditioned media by centrifugation. The resulting supernatant containing the secreted factors was used immediately or frozen at −80°C until use.

Mitochondrial ROS analysis

Mitochondrial ROS production was analyzed in MSC by staining the cells with Mitosox Red Mitochondrial Superoxide Indicator (ThermoFisher Scientific) according to the manufacturer's instructions. The stained cells were analyzed by flow cytometry and mean fluorescent intensity was calculated for each condition.

Phospho protein analysis

The phosphorylated form of NFκB was analyzed by flow cytometry. The cells were fixed with 2% formaldehyde, permeabilized with 100% ice-cold methanol and incubated with anti-phospho NFκB antibody (pS529, BD Biosciences) for 1 h at room temperature. The cells were washed and analyzed with FACS calibur.

RNA extraction and real-time PCR

The cell pellet was resuspended in TriZol reagent (Thermo Fisher Scientific) and total RNA was extracted using TRIzol Plus RNA Purification Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The resulting RNA was reverse transcribed using high capacity cDNA synthesis kit and oligodT primers at 37°C for 120 min. Real-time PCR was performed using Power SyBr Green reagents in an ABI 7500 system (Thermo Fisher Scientific). The gene expression levels in each sample was normalized to their respective GAPDH expression levels. The fold change in the expression levels compared to the control was calculated using $2^{-\Delta\Delta Ct}$ method.

Quantification of secreted IL6 level

IL6 secreted levels in the conditioned media of MSC subjected to various conditions were determined using Human IL6 ELISA Kit (Thermo Fisher Scientific) as per the manufacturer's instructions. MSC were treated with inhibitors or co-cultured with K562 cells for the indicated time period. Fresh serum free media was added to the cells and incubated for 24 h at 37°C, the conditioned media was collected, centrifuged and secreted IL6 levels were determined.

Exosome depletion

Exosome release in the MSC were blocked by treatment of MSC with 10μm of GW4869 (Sigma) overnight in serum free media as described earlier (Kang et al. 2015). The media was replaced with fresh media and conditioned media was collected after 6 h and frozen at −80°C until required.

Data analysis

Flow cytometric data was analyzed using FlowJo software. Statistical analysis was performed with SPSS software and values of $p < 0.05$ were considered statistically significant. The comparison between different treatment groups was done using paired samples T test and patient samples from different groups was analyzed by Mann-Whitney non-parametric variables test.

Results

CML bone marrow derived MSC show functional defects

In hematologic malignancies such as CML, the cancer cells utilize the signals from bone marrow microenvironment to proliferate and acquire chemoresistance. In order to understand the changes incurred by the stromal cells during CML, mesenchymal stem cells were isolated from bone marrow of CML patients at the chronic stage during diagnosis. CML derived MSC (CML-MSC) were spindle shaped, could be passaged in culture up to 8 passages and showed normal CFU-F ability (26.5 ± 4.5 colonies per 100 cells seeded). When induced with specific differentiation media, CML-MSC differentiated into adipocytes, chondrocytes and osteoblasts (Fig. 1a, b). However, they showed a significantly reduced osteogenic differentiation potential (Fig. 1b-d) compared to control MSC as determined by alizarin red staining and *OSTEOCALCIN* expression. To determine whether the reduced osteogenic differentiation of CML-MSC was due to its interaction with the CML cells, control-MSC were co-cultured with CML cell line K562, their gene expression and differentiation potential was analyzed. When control-MSC were co-cultured with K562 cells, there

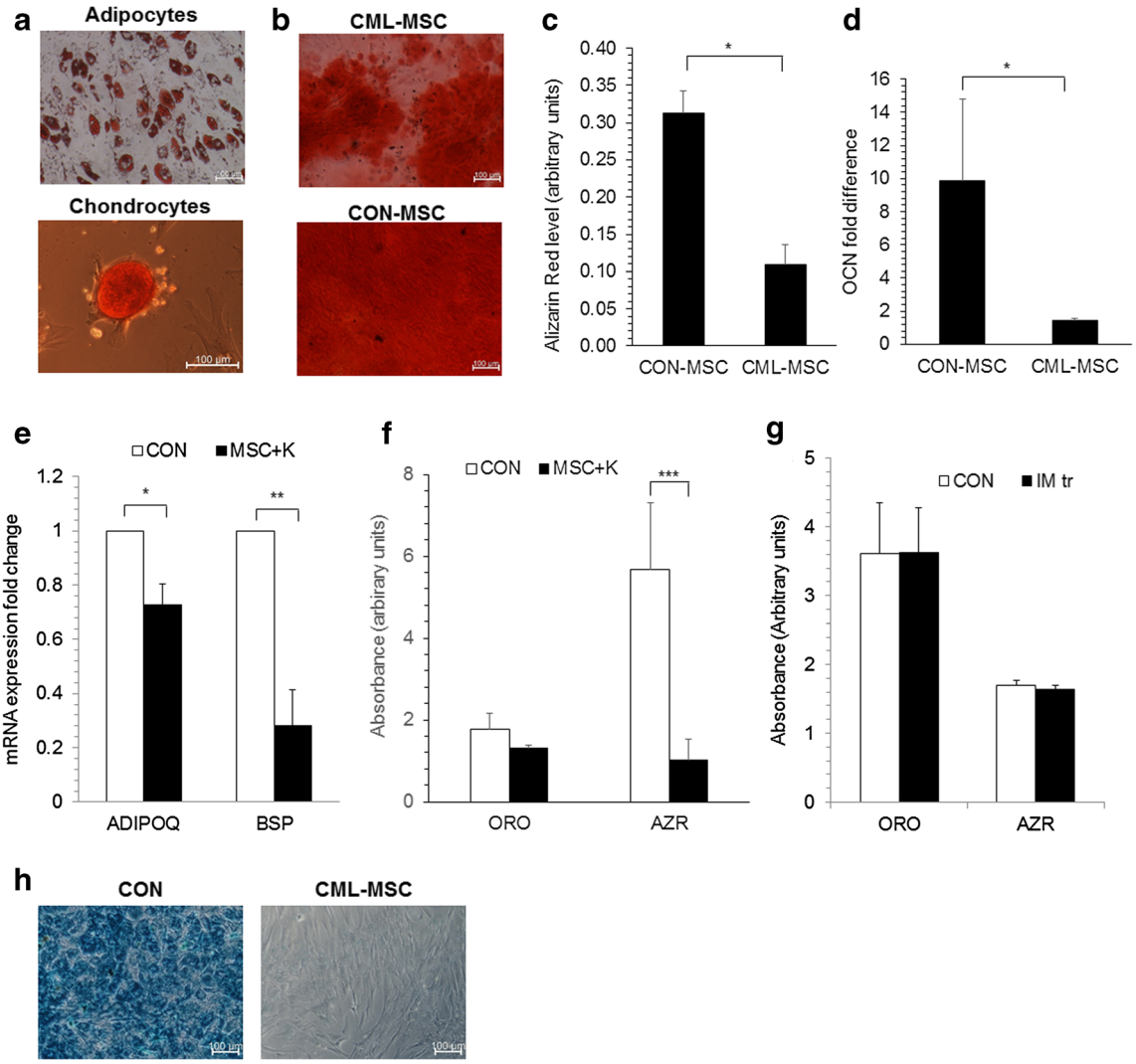


Fig. 1 CML derived MSC have reduced osteogenic differentiation potential. **a** MSC isolated from bone marrow of CML patients (CML-MSC) were differentiated into adipocytes and chondrocytes. Adipogenic differentiation was determined by Oil Red O staining and chondrogenic differentiation by Safranin-O staining. **b-d** CML-MSC and control-MSC (CON-MSC) were differentiated into osteoblasts and stained with alizarin-Red. **b** Microscopic image showing osteoblasts derived from CML-MSC and CON-MSC stained with alizarin red. **c** Alizarin red levels or (**d**) *OSTEOCALCIN* (OCN) transcript levels in osteo differentiated CON-MSC and CML-MSC. **e**, **f** Control-MSC were co-cultured without (CON) or with K562 cells for 72 hours (MSC + K) and (**e**) expression levels of ADIPOQ and BSP were determined by real-time PCR. **f** Control-MSC (CON) and MSC co-cultured with K562 cells (MSC + K) were subjected to adipogenic and osteogenic differentiation by addition of induction media. Adipogenic differentiation was determined by oil red O (ORO) staining and osteogenic differentiation was detected by alizarin red staining (AZR). ORO and AZR staining in individual samples were quantified colorimetrically. **g** Control-MSC were treated without (CON) or with imatinib (10 μM) for 48 h (IM tr) and subjected to adipogenic and osteogenic differentiation. Adipogenic, osteogenic differentiation was detected by ORO, AZD staining respectively and quantified. **h** Representative microscopic image showing control late passage MSC (CON) and CML-MSC stained histochemically for β-galactosidase activity. Blue stain represents the senescent cells. Values are mean ± SE, $*p < 0.05$, $**p < 0.005$, $***p < 0.0005$, $n \geq 3$

was a significant reduction in the expression levels of adipogenic marker gene *ADIPOQ* and osteogenic gene *BSP* prior to differentiation induction (Fig. 1e). When subjected to directed differentiation, the K562 co-cultured control-MSC showed significantly reduced differentiation into osteoblasts similar to that observed in the CML patient derived MSC (Fig. 1f). However, IM treatment which is the first line of therapy for CML did not affect the adipogenic and osteogenic differentiation potential of MSC (Fig. 1g). CML-MSC in culture did not show any senescent phenotype when stained for β-galactosidase activity (Fig. 1h).

CML cells modify the cell surface phenotype of MSC

The cell surface antigen expression profile of CML-MSC were similar to the control-MSC, however, they showed significantly reduced expression levels of CD13, CD73 and CD90 (Fig. 2a). To test further, when conditioned media from

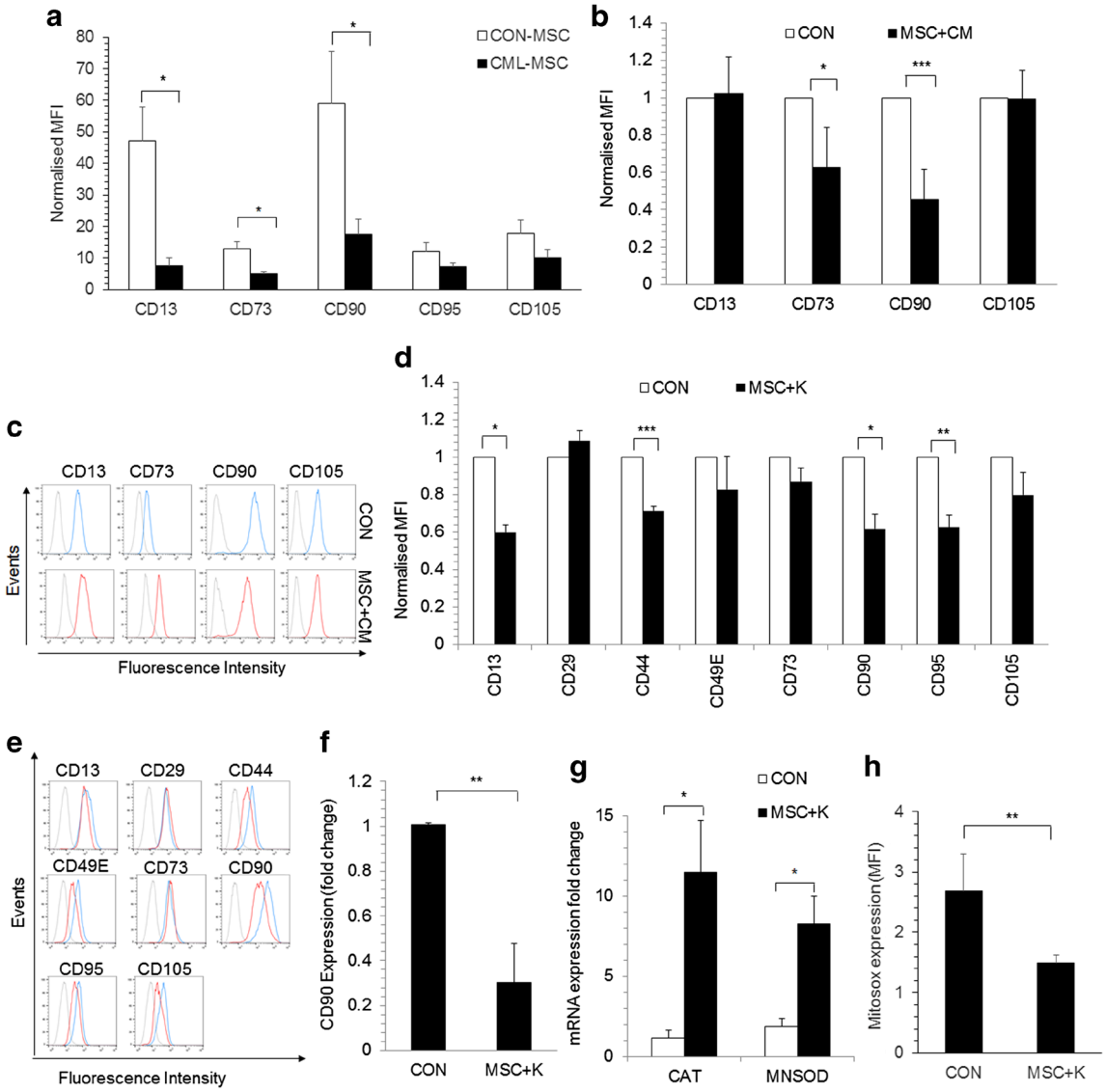


Fig. 2 Interaction with K562 CML cells and its paracrine factors modified cell surface antigen expression in MSC. **a** Cell surface expression of CD13, CD73, CD90, CD95 and CD105 in control-MSC (CON-MSC) and CML-MSC was analyzed by flow cytometry. Mean (geometric) fluorescent intensity (MFI) was calculated for each marker against its isotype control. **b**, **c** Control-MSC were cultured in conditioned media derived from K562 cells for one week and their cell surface gene expression in control-MSC (CON) and conditioned media treated MSC (MSC + CM) was analyzed by flow cytometry. MFI of analyzed markers was normalized to control-MSC. **c** Representative flow cytometry histogram showing cell surface antigen expression levels in CON and MSC + CM conditions. Grey line represents the isotype control, blue and red line represents the stained cells. **d**, **e** Control-MSC were co-cultured without (CON) or with K562 cells (MSC + K) for one week and their cell surface gene expression profile was determined by flow cytometry. MFI was calculated for each antigen and normalized to control-MSC. **e** Representative flow cytometry histograms showing the cell surface antigen expression in CON (blue line), MSC + K (red line) and isotype control (grey line). **f**, **g** RNA was extracted from control-MSC (CON) and K562 co-cultured MSC (MSC + K) and reverse transcribed into cDNA. mRNA expression levels of (**f**) CD90, (**g**) CAT and MnSOD was analyzed by real-time PCR. (h) Mitochondrial ROS levels in CON and MSC + K were analyzed by staining with mitosox red and MFI of mitosox was normalized to unstained cells. Values are mean ± SE, *p < 0.05, **p < 0.005, ***p < 0.0005, n ≥ 3

K562 cells were added to the control-MSC, there was a significant reduction in the cell surface expression levels of CD73 and CD90 in conditioned media treated control-MSC compared to the untreated cells (Fig. 2b, c). On the other hand, when cultured in direct contact with K562 cells, the co-cultured MSC showed downregulated cell surface expression of CD13, CD44, CD90 and CD95 (Fig. 2d, e). When tested further, the reduction in CD90 expression in MSC during co-culture with CML cells was also observed at the transcript level (Fig. 2f). To understand whether CML cells induce oxidative stress on MSC, the transcript levels of ROS scavenging enzymes MnSOD and CAT was analyzed in MSC co-cultured with K562 CML cells. There was a significant increase in transcript levels of ROS scavenging enzymes

MnSOD and CAT (Fig. 2g) which correlated with the reduced ROS levels in these cells (Fig. 2h).

CML cells modify stromal cells for chemoprotection

We further found that CML derived MSC had high IL6 mRNA levels compared to the control-MSC (Fig. 3a). Similarly, interaction of control-MSC with K562 cells resulted in increased expression of IL6 and TNFα mRNA in the stromal cells (Fig. 3b). When MSC were co-cultured with the K562 cells, they secreted significantly high levels of IL6 post exposure to the leukemic cells. This increase in IL6 was mediated by an increased phosphorylation of NFκB in MSC during its interaction with the CML cells (Fig. 3c, d). Treatment of MSC with TNFα significantly induced IL6 secretion whereas addition of NFκB inhibitor BAY 11–7082, downregulated the TNFα induced IL6 secretion (Fig. 3e). In the same way, when K562 co-cultured MSC were treated with BAY 11–7082, secreted IL6 level was downregulated (Fig. 3f, Suppl. File 1). IL6 levels increased when MSC were differentiated into osteoblasts, but IL6 levels did not predict osteogenic differentiation ability of MSC (Fig. 3g, h).

Since interaction with CML cells significantly increased NFκB phosphorylation and IL6 secretion by MSC, we tested to see whether factors from MSC could chemoprotect K562 cells from IM treatment. When K562 cells were treated with IM in the presence of conditioned media from control-MSC, there was a significant reduction in the percentage of apoptotic cells compared to the cells treated with IM without the conditioned media (Fig. 4a). Since we found that IL6 secreted levels in MSC increased post co-culture with the K562 cells, we tested the effect of IL6 receptor (IL6R) blocking in chemoprotection against IM. K562 cells incubated with MSC derived conditioned media were treated with IL6R blocking monoclonal antibody tocilizumab during treatment with IM. IL6R blocking did not chemosensitize K562 cells to IM treatment, when conditioned media from MSC were present. Similarly, blocking the release of exosomes into the MSC derived conditioned media did not affect the chemosensitivity of K562 cells. However, when conditioned media collected from MSC co-cultured with K562 cells was added, IM induced apoptosis was further inhibited in K562 cells. (Fig. 4a, b).

Thus, K562 CML cells obtained survival advantage by modifying the differentiation ability and gene expression in the microenvironment stromal cells.

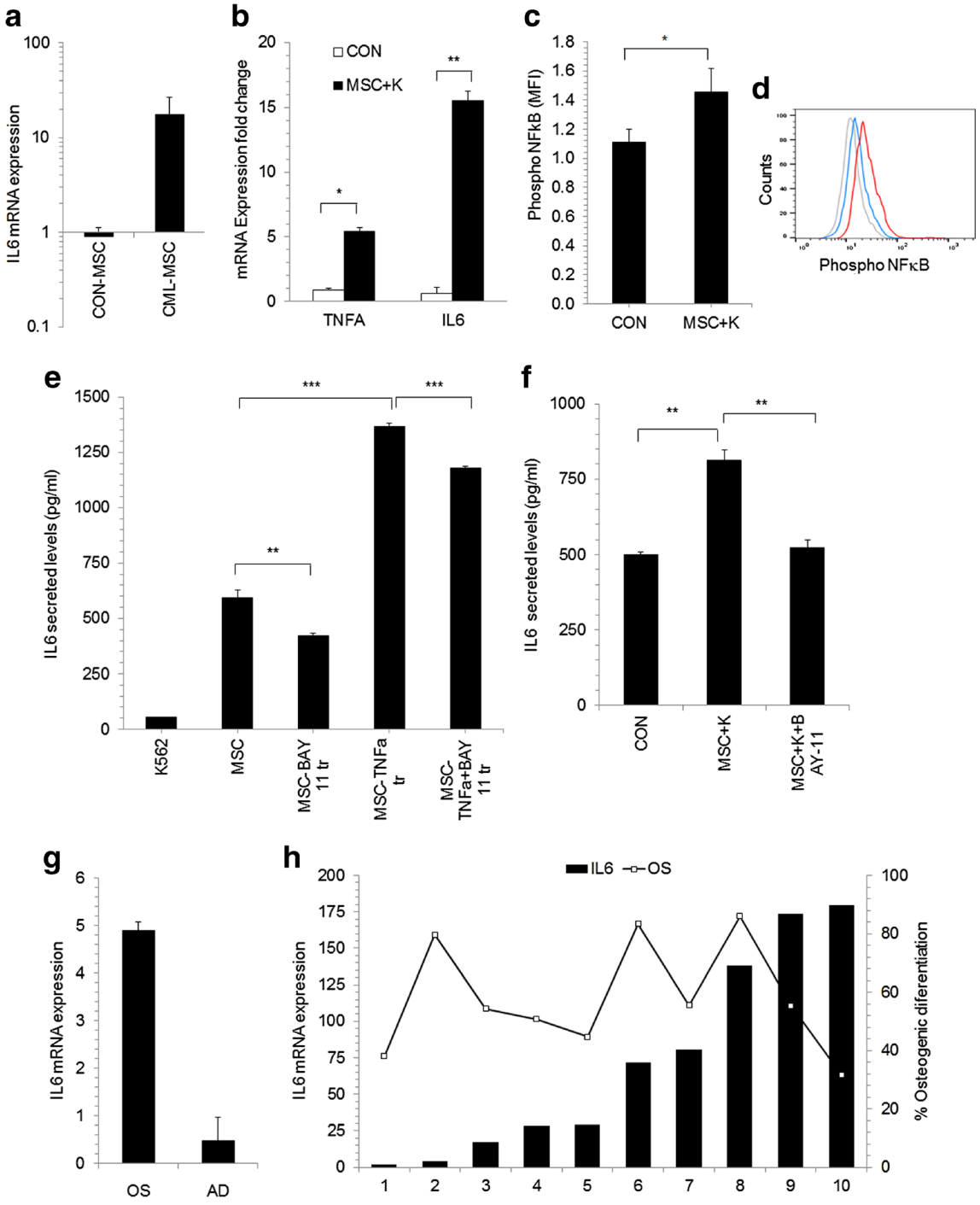
Discussion

In the current study, we investigated the effect of CML cells on the stromal cells of the BM microenvironment. An important role for BM stromal cells in leukemia progression,

Fig. 3 IL6 expression in MSC was induced through contact with K562-CML cells. **a** IL6 transcript levels were determined in control-MSC (CON-MSC) and CML-MSC by real-time PCR. **b** Control-MSC were co-cultured without (CON) or with K562 cells (MSC + K) and mRNA levels of TNFα (TNFA) and IL6 was determined by real-time PCR. **c** Control-MSC were co-cultured with K562 and phosphorylated NFκB levels was determined by flow cytometry in control (CON) and co-cultured (MSC + K) MSC. **d** Representative flow cytometric histogram showing the phospho NFκB analysis in CON (blue line) and MSC + K (red line). Grey line in the histogram is isotype control. **e** Secreted IL6 levels was determined by ELISA in conditioned media collected from K562 cells (K), control-MSC (CON), MSC treated with NFκB inhibitor BAY 11–7082 (10μM; MSC + BAY 11tr), MSC treated with TNFα (10 ng/ml; MSC-TNFa tr) or MSC treated with both TNFα and BAY 11–7082 (MSC-TNFa + BAY 11 tr). **f** Conditioned media was collected from control-MSC (CON), MSC co-cultured with K562 cells for 48 hours (MSC + K) or MSC treated with NFκB inhibitor BAY 11–7082 during co-culture with K562 cells (MSC + K + BAY 11). Secreted IL6 levels was determined by ELISA. Represented IL6 levels are per 1×10^5 cells/ml. **g** MSC were differentiated into adipocytes (AD) or osteoblasts (OS) for one week and IL6 mRNA expression level was determined by real-time PCR. **h** IL6 mRNA expression level was determined in MSC isolated from 10 different bone marrow donors. The respective MSC were differentiated into osteoblasts and their percentage of osteogenic differentiation was determined by alkaline phosphatase staining. The x-axis represents the different donors and bar shows the IL6 expression levels in different MSC on the primary y-axis and the line represents the osteogenic differentiation percentage in the respective MSC in the secondary y-axis. Values are mean ± SE, *p < 0.05, **p < 0.005, ***p < 0.0005, n ≥ 3

relapse and chemoresistance has been documented by several studies. MSC from hematologic malignancies have been shown to have functional abnormalities such as reduced osteogenic differentiation in multiple myeloma (Xu et al. 2012), acute myeloid leukemia (Geyh et al. 2016), increased osteogenesis in primary myelofibrosis (Martinaud et al. 2015) and increased adipogenic differentiation in acute lymphoblastic leukemia (Lopez et al. 2014). In our current study, we showed that MSC isolated from CML patients had reduced osteogenic differentiation and this was confirmed during co-culture with K562 cells. We found that the interaction of MSC with the leukemic cells down regulated the expression of osteogenic marker BSP and reduced their osteogenic differentiation potential. However, imatinib, which is a protein tyrosine kinase inhibitor used to treat CML, did not have any significant effect on the osteogenic and adipogenic differentiation of MSC. Osteoblasts are important components of the bone marrow niche to maintain HSC (Calvi et al. 2003; Zhang et al. 2003), and reduction in osteoblast number or dysfunctional osteoprogenitor cells were less supportive of normal hematopoiesis and more supportive of malignant hematopoietic cells (Kode et al. 2014; Krevvata et al. 2014; Raaijmakers et al. 2010). Thus, a leukemia induced reduction in osteogenic differential potential of MSC may result in BM microenvironment which is hostile for normal HSC but conducive to the leukemia cells proliferation.

Moreover, interaction with the leukemia cells also modified the cell surface phenotype of MSC and cell surface



expression represents functional characteristics. MSC isolated from patients with hematologic malignancies expressed low CD90, which correlated with their reduced immunomodulatory properties (Campioni et al. 2009; Jaganathan et al. 2010). In our study, MSC isolated from CML patients showed reduced CD13, CD73 and CD90 expression. However, reduction in CD90 expression was also observed when leukemia cells derived conditioned media or leukemia cells were added to the MSC culture. A significant decrease also in transcript level of CD90 was observed in MSC co-cultured with the leukemia cells. With several studies suggesting the role of CD90 in cancer progression (Kumar et al. 2016), CD90 expression in stromal cells might be an important molecule to study in detail to understand its importance in leukemia progression and chemoresistance. MSC co-cultured with K562 cells also showed reduced expression of CD44, an adhesion molecule that is important for normal hematopoiesis.

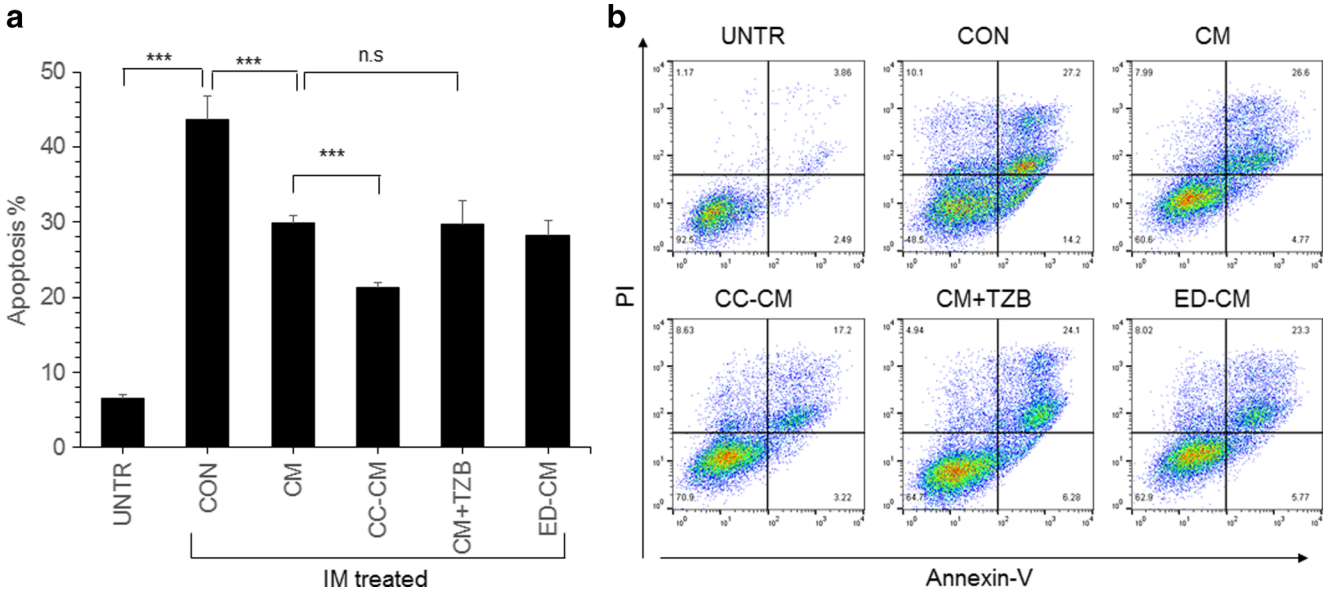


Fig. 4 Paracrine factors from MSC which were in contact with K562 cells chemoprotected K562 cells from imatinib induced cell death. **a** K562 cells were left untreated (UNTR) or treated with IM alone (CON), or in the presence of control-MSC derived conditioned media (CM), conditioned media from control-MSC co-cultured with K562 cells (CC-CM), tocilizumab along with CM (CM + TZB) or exosome depleted conditioned media from MSC (ED-CM). Apoptosis in treated K562 cells was analyzed by annexin-V/PI staining after 48 hours. **b** Representative flow cytometric dot plots showing apoptosis in different conditions as shown in (**a**). Values are mean ± SD, ***p < 0.0005, n ≥ 3; n.s-not significant

An aberrant expression of cytokines was reported in acute myeloid leukemia, multiple myeloma and in some cases of lymphoma (Arnulf et al. 2007; Huang et al. 2015; Wallace et al. 2001). Schmidt et al. identified a high expression of angiogenic cytokines such as VEGF, PlGF, IL6 in the BM of CML mouse (Schmidt et al. 2011). High levels of IL6 along with transforming growth factor-α (TGFα) were detected in the plasma of CML patients (Nievergall et al. 2014) and high levels of TNFα and IL6 were reported in BM plasma of BCR-ABL mouse providing a preferential growth advantage to LSC (Zhang et al. 2012). Similarly, our study found significantly high levels of IL6 mRNA expression in MSC isolated from CML patients and in MSC co-cultured with K562 cells which might have immune modulatory functions as also reported by others (Giallongo et al. 2016). Even though MSC isolated from CML patients were separated from the leukemia cells and cultured alone, the IL6 expression levels remained significantly higher than the control cells, suggesting sustained aberrations in gene expression. Schmidt et al. reported NFκB activation to be responsible for secretion of inflammatory cytokines by MSC (Schmidt et al. 2011) and similarly in our study, an increased phosphorylation of NFκB was identified in MSC co-cultured with CML cells which led to increased IL6 secretion. Increased IL6 secretion through NFκB activation was confirmed by treatment with NFκB inhibitor BAY 11–7082 which brought the IL6 amount to basal levels. This increased NFκB activation might be responsible for reduced osteogenic differentiation (Chang et al. 2013) of CML-MSC

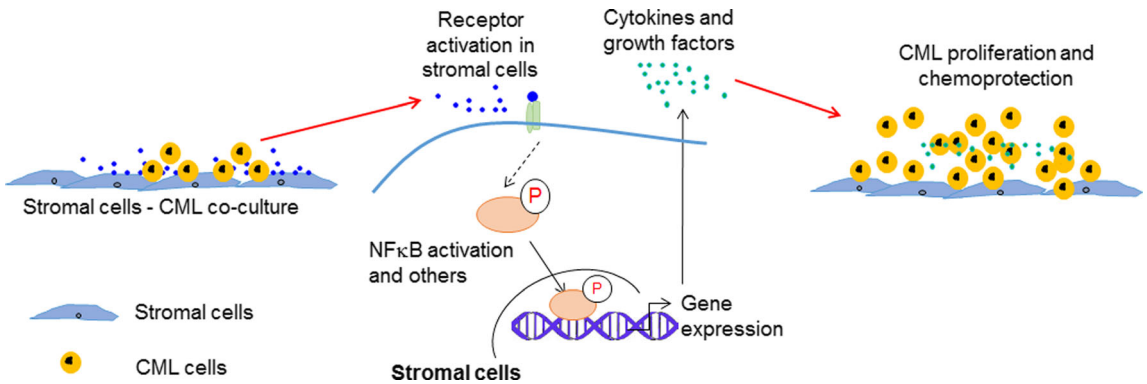


Fig. 5 Figure showing the interaction between leukemia cells and stromal cells resulting in aberrant expression of cytokines, growth factors by the stromal cells which might help in CML proliferation and chemoprotection

and MSC interacting with K562 cells. Although increase in IL-6 mRNA levels during osteogenesis was found in our study, no significant correlation was observed between the IL-6 mRNA levels and osteogenic differentiation potential of MSC as reported by others (Pricola et al. 2009).

The conditioned media from CML cells was sufficient to offer survival advantage against IM induced cell death. Although high IL6 levels were seen in conditioned media of CML-MSC, inhibition of IL6 receptors (secreted and cell surface bound) through the monoclonal antibody tocilizumab even at high concentrations did not chemosensitize CML cells to IM treatment. Saini et al. reported that the pro-survival effects offered by IL6 did not depend on IL6 receptor expression but rather on iNOS pathway (Saini et al. 2014) which might explain the lack of apoptotic effect that was observed when IL6 receptor was inhibited in CML cells in our study. Also, the chemoprotective effect of MSC derived conditioned media were not mediated through exosomes but might be through FGF2 as reported by Traer et al. (Traer et al. 2014). Taken together, interaction of leukemia cells with the stromal cells modifies the stromal cells by activating NFκB pathway and its osteogenic differentiation potential, which might support CML cell proliferation and chemoprotection (Fig. 5). Cross-talk between cancer cells and stromal cells which facilitated cancer cell proliferation and chemoresistance development have been reported by several studies (Geyh et al. 2016; Kumar et al. 2017; Schroeder et al. 2016). As a result of this reciprocal interaction, the stromal cells showed aberrant gene expression profiles and leukemia cells acquired proliferative advantage and chemoprotection. Thus, utilizing a combinatorial therapeutic approach to target stromal cells to abolish the aberrant cross-talk between the leukemia cells and stromal cells might lead to better patient outcome.

Acknowledgements This work was supported by grants from Indian Council of Medical Research (ICMR) to BGJ, JB and Department of Biotechnology (DBT) to BGJ. ICMR, DBT did not have any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contribution Conceived and designed the experiments: AK JB BGJ. Performed majority of experiments: AK. Performed experiments: TA AS. Analyzed the data: AK BGJ. Wrote the manuscript: AK JB BGJ. All authors have read and approved the final version of the manuscript.

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