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Mini review

Mesenchymal stem cells in myelodysplastic syndromes, contributions to disease pathogenesis and biomarker discovery

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Key words: mesenchymal stem cells (MSCs), myelodysplastic syndromes (MDS), haematopoietic stem and progenitor cells (HSPCs), bone marrow microenvironment, biomarker discovery

Abstract

It is widely accepted that alterations in both haematopoietic stem cell compartment and the bone marrow microenvironment contribute to the pathogenesis of myelodysplastic syndromes. In order to gain insights into disease pathogenesis, a main cell type in the bone marrow microenvironment, mesenchymal stem cells, have been studied with great interest in the recent past. These cells are multipotent stem cells having the ability to differentiate in to other main resident cell types of the bone marrow such as chondrocytes, adipocytes and osteogenic cells. Their properties of plastic adherence in cell culture and characteristic cell marker expression have enabled easy isolation, characterization and used in research studies. These cells are known to interact closely with the haematopoietic stem and progenitor cells to maintain normal haematopoiesis. Their interactions are tightly regulated via many mechanisms including immunomodulation. Research studies have been done on marrow derived mesenchymal stem cells by many groups. Conflicting results regarding cell morphology, cell culture characteristics, cell proliferation and karyotype have been found. Altered haematopoietic support, immunomodulation, increased cellular senescence and genomic changes indicate that deranged mesenchymal stem cells as playing a definitive role in disease pathogenesis while providing leads to biomarker discovery in myelodysplastic syndromes. Preliminary studies on intercellular communications between mesenchymal stem cells and haematopoietic cells via extracellular vesicles have shown how mesenchymal stem cells could bring about myelodysplastic changes in the marrow. Current hypomethylating and immunomodulatory therapy were shown to have effects on stromal cells. Further studies on mesenchymal stem cells are warranted.

Introduction

The bone marrow microenvironment (BMM) is a complex dynamic structure formed by non-haematopoietic cells, extracellular matrix constituents, growth factors, hormones, etc. having vascular and neural supply[1]. It is modulated by stimuli arising due to physiological and pathological processes such as aging, infections, and malignancies[1]. The haematopoietic stem and progenitor cells (HSPCs) residing in the haematopoietic stem cell (HSC) niches within the BMM are supported and regulated by constituents of the BMM[1,2]. The mesenchymal stem cell (MSC) is a main BMM constituent cell, first described by Friedenstein in 1970s as "fibroblast colony-forming cells" based on their fibroblast like morphology and their ability to form colonies in vitro, and were later named as mesenchymal stem cells (MSCs) by Pittenger and other groups, based on their ability to differentiate into cells derived from embryonic mesoderm[1]. The international society for cellular therapy (ISCT)

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designated the cells as multipotent mesenchymal stromal cells; however retained the acronym MSC[3]. In order to harmonize research work, ISCT proposed minimal criteria to define MSCs:

(1) Adherence to plastic in standard cell culture conditions

(2) Expression of surface antigens; CD105, CD73 and CD90 positivity (=>95%) and negativity (=<2%) for CD45, CD34, CD14/CD11b, CD79α/CD19 and HLA-DR

(3) Multipotent differentiation potential to osteoblasts, adipocytes and chondroblasts. Cells meeting these criteria were termed as mesenchymal stem cells (MSCs)[21].

The review will use the acronym MSCs or the term mesenchymal stromal cells based on the work of the citing literature. The MSCs play a crucial role in HSPC survival, proliferation and differentiation and has immunomodulatory functions and play a key role in overall BM homeostasis[1,2]. Seminal work and later studies on BMM and HSCs have shown that both HSPCs and MSCs interact with each other at stem cell level and their functions are tightly regulated within the BMM[2,4].

Myelodysplastic syndrome (MDS) is a HSC disorder, originating from Lin-, CD34+, CD38- and CD90+ haematopoietic lineage cells[5]. Research show that MSCs are deranged in MDS, based on in vitro studies on MSCs isolated from the BM (BM-MSCs); and in vivo MDS-murine model studies[2,6]. Myelodysplastic syndrome is a pre-leukaemic disorder, and research has shown changes in MSCs linking to initiation, progression, and therapy outcomes in acute myeloid leukaemia (AML) and therefore a potential therapeutic target in AML[7].

This mini review aims to highlight research work carried out on MDS-MSCs within the last two decades. Research studies analysing MDS-MSC morphology, proliferation, differentiation, immune modulation, and genomic profiles as well as study findings of extracellular vesicles (EVs) derived from MDS-MSCs and effects of standard MDS therapy on MSC are included in this review. The findings will be discussed along with their clinico-pathological correlations.

Morphological and biological alterations in MDS-MSCs and their pathological and clinical implications in MDS

A large-scale study had been done on newly diagnosed untreated MDS-BM-MSCs derived from patients having MDS-World Health Organization (WHO) 2008 subtypes: refractory cytopenia with multilineage dysplasia (RCMD), refractory anaemia with excess blasts (RAEB), deletion 5q (5q-), MDS unclassified (MDS-u) and secondary AML with MDS related changes (n=106)[8]. The study findings reported that in comparison to healthy-control-MSCs, the BM-MSCs showed altered morphology (larger forms) and cell cultures showing disorganized cell growth, low proliferation and poor osteoblastic differentiation, with down regulation of *Dicer-1* (codes for an endonuclesase required for microRNA (miRNA) synthesis). Previous murine studies[9] had shown that *Dicer-1* gene deletion in osteoblasts had resulted in ineffective haematopoiesis and MDS phenotype. The latter had helped to validate the former experimental findings.

Ferrer et al[10] had studied BM-MSCs of MDS patients (n=20, study groups: 5q-, "lower risk" and "higher risk" using the international prognostic scoring system -IPSS) and reported changes in MSCs in all the groups in comparison to normal-control-MSCs. Changes included morphological changes, disorganized growth in culture, reduced cell proliferation and differentiation potential, up-regulation of expression of adhesion molecules (CD146 and CD166); adhesion molecules facilitate cross-talk between HSPCs and the stromal compartment; and down regulation of messenger ribonucleic acid expression of key HSPC supportive cytokines (stromal cell-derived factor-1α, stem cell factor (SCF) and angiopoietin-1). The researchers interpret these changes to indicate reduced HSPC support by MSCs and deranged cell mobility patterns. Lenalidomide pre-treatment of the MDS-MSCs had modulated MSC phenotypes and HSC support, differentially in the sub-groups thus indicating a therapeutic benefit of the immuno-modulatory agent on MDS-MSCs.

Zhao et al[11] reported their study findings following culture expansion of BM-MSCs (n=14, MDS-WHO

subtypes: refractory anaemia (RA), RAEB-1, RAEB-2, and 5q-) as showing cell morphology and ultrastructure, adipogenic and osteogenic differentiation, cell surface marker expression and growth kinetics similar to normal-control-MSCs, but impaired HSCs support and immunoregulatory functions including increased expression of interleukin (IL)-6 compared to controls. They also reported decreased expression of SCF similar to Ferrer et al.[10] Corradi et al.[12] from their study which included low to high grade MDS-BM-MSCs (n=26) reported normal cellular morphology, differentiation ability and cell marker expression, but reduced cell proliferation in comparison to healthy-donor-MSCs, and the group postulated that the latter finding could indicate an intrinsic MSC defect.

Boada et al[13] in their BM-MSCs study (untreated patients, n=35 versus normal-control-MSCs, n=22) had shown low proliferation, changed morphology (larger cells with less ramifications), higher production of IL-6, IL-17A, interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α) in comparison to normal controls. They had also studied the effects of the hypomethylating agent 5-azacytidine (5-Aza) on some of the patients in the group (MDS-IPSS low risk (LR) and intermediate-1 risk, n=10 versus normal-control-MSCs (n=10) and showed that 5-Aza treatment did not change cell cytotoxicity between the groups. However, 5-Aza was able to suppress IL-6 secretion in the MDS-MSCs and change their morphology to more rounded cells. Wenk et al[14] had also investigated the effects of epigenetic therapy of 5-Aza on BM-MSCs (untreated patients, n=10 versus normal-control-MSCs, n=10) and report lack of reversal of poor differentiation (osteogenic and chondrogenic) in MSCs following treatment. However, enhancement of HSPC supportive function had been demonstrated. Taken together, these studies could indicate that current MDS epigenetic therapy with 5-Aza modify derangements in pathways related to immunomodulation and HSC supportive functions mediated via MSCs as well as reverse intrinsic MDS-MSC cellular derangements to some extent.

Kode et al[15] in a study of MDS and AML patients (n=107; MDS-WHO subtypes: refractory anaemia with ring sideroblasts (RARS), refractory cytopenia with unilineage dysplasia, refractory cytopenia with multilineage dysplasia (RCMD), RAEB-1 and RAEB-2 and primary AML and AML secondary to MDS) versus healthy-control-MSCs (n=56) had shown, increased nuclear β-catenin accumulation (β-catenin stimulates expression of Jagged-1 Notch Ligand) in osteoblasts. Reversal of β-catenin expression had resulted in improvement of anaemia and myeloid expansion. Thus, BMM is a potential therapeutic target in MDS. Johnson et al [16] following comparative analysis of CD271+ BM-MSC density in BM core biopsies in MDS patients (n=64) versus benign cytopenia patients, had shown that CD271+ cell density correlated with higher grade MDS and poorer prognosis; and suggests CD271+ MSCs as an independent prognostic marker for MDS.

Many other studies have demonstrated biological and immunological alterations in MDS-MSCs providing evidence to support a pathogenic role of MDS-BM-MSCs and its potential as a therapeutic target to develop novel therapies. Pleyer et al[2] has extensively reviewed these studies describing biological alterations, and mechanisms of immune activation, suppression and immune evasion by MSCs and their progenitors in MDS along with AML and their clinical translations.

Cell senescence in MDS-MSCs and biological and therapeutic implications

Impaired growth kinetics of MSCs has been attributed to increased MSC cellular senescence, detected by increased presence of senescence-associated-beta-galactosidase (SA-β-Gal); the gold standard for detecting cellular senescence in cells[8]. Zhao et al[17] having studied MDS-BM-MSCs of a large MDS cohort (n=>70, MDS-WHO subtypes: RA, refractory anaemia with ring sideroblasts (RARS), RCMD, RCMD-RS, RAEB-1, RAEB-2 and MDS-u and based on IPSS low/intermediate-1 indicated as "lower risk" and intermediate-2/high as "higher risk") reported detection of SA-β-Gal in all MDS subtypes but higher in the lower risk group. Furthermore, senescence was associated with lower adipogenic and osteogenic differentiation potential. They had postulated that changes in the biological properties vary among MDS subtypes

and senescence could contribute to ineffective haematopoiesis. The same group[17] had shown reduced *Dicer-1* gene expression in both risk groups and recovery of cell senescence and improvement of osteogenic and chondrogenic differentiation following transfection of MSCs with *Dicer-1* gene; taken together with other studies[8,9] Dicer-1 is a promising target in MDS diagnosis and therapy. Many other research groups had reported deranged cytokine secretion (pro-inflammatory cytokines TNF-α, IL-6, IL-8 etc.), aberrant cellular signaling and increased accumulation of reactive oxygen species (ROS) and reduced HSC supportive functions in association with cell senescence in MDS-MSCs and they have been recently reviewed[18]. This review discusses the potential of hypomethylating agents such as 5-Aza, anti-oxidant agents and other molecules in reversing cell senescence in MDS-MSCs.

Chromosomal aberrations in MDS-MSCs and pathogenesis of MDS

Literature reports conflicting evidence regarding chromosomal aberrations in MDS-BM-MSCs. In their study of BM-MSCs (n=22, MDS WHO subtypes: RA, RCMD, RAEB-1, RAEB-2 and 5q-) Song et al[19] had shown overall chromosomal aberrations being 68%, including random loss of chromosomes, which they related to MDS chromosomal instability. Interestingly, 5q- patien's MSCs had not carry 5q-. Xiong et al[20] had reported chromosomal aberrations in 30% of BM-MSCs in a Chinese MDS cohort (n=30) with gains, losses and deletions including 5q- in MDS-RARS subtype. Chromosomal studies followed by fluorescent in-situ hybridization and array comparative genomic hybridization allowing in depth study of chromosomal abnormalities and genomic imbalances, were done by Lopez-Villar et al[21] on BM-MSCs (n=36, MDS-WHO subtypes: 5q-, RA, RARS, RCMD-RS, RAEB-1, RAEB-II, MDS-u) and had demonstrated distinct cytogenetic abnormalities to that of their BM counterparts. Important detections were gain of genetic material in areas containing genes implicated in cell-adhesion and tumour-genesis, and a genomic profile specific to 5q- subtype (7p22.3, 19p13.3, 19p13.11). Thus, the changes implicate MSCs in MDS pathogenesis, with special emphasis on 5q- subtype. Similarly, Corradi et al[12] had not found corresponding aberrations in BM-MSCs to their BM karyotypes. In contrast to others, Zhao et al[11] report normal karyotypes in both freshly sorted and cultured (up to 10 passages) BM-MSCs. The MDS patients' counterpart BM had shown cytogenetic abnormalities. Based on theirs and other similar research findings[11,12,19-22] the following postulations had been made regarding the pathogenic basis of MDS based on the karyotype: MDS could originate via two malignant cell clones instead of a single common malignant precursor cell (based on presence of distinct cytogenetic abnormalities in BM-MSCs and counterpart BM mononuclear cells) or BM-MSCs are not clonal (in the absence of chromosomal aberrations in BM-MSCs). Furthermore, they mention inherent MSC genomic instabilities and in vitro MSC culture conditions could affect cytogenetic test results. Therefore, further research is warranted to make definitive conclusions.

Genomic changes in MDS-MSCs and their pathological and clinical significance

Variant analysis on nucleic acids, methylation pattern analysis and miRNA profiling had provided valuable insights into MDS pathobiology at genomic level. Some of the research findings are mentioned below.

Azuma et al[22] had reported synonymous and non-synonymous variants in MDS-MSCs following DNA sequencing using a 50 gene myeloid panel. Despite ≥500 read depths, due to small samples size (n=5) and low variant allele frequency, specific inferences were not made. Seminal studies on DNA methylation on MDS-MSCs by Geyh et al.[8] on a carefully selected age and sex matched samples from MDS-WHO subtypes: RCMD, RAEB, normal-control-MSCs (n=12 out of n=106 cohort), reported highly specific gene methylation patterns in MDS subtypes which corresponded to gene expression and biological functions MDS-MSCs. Bahgat et al[23] had studied the methylome (i.e. genomic distribution of methylated cytosines) in MDS-BM CD45- stromal cells from 5-Aza treated and untreated patients versus healthy-control-MSCs. The group reported aberrant hypermethylation in the former cohort; latter two had showed similar results. Furthermore, hypermethylation had

shown to affect cellular pathways controlling cell morphology, signaling and transport; including wnt/β-catenin pathway and integrin signaling pathways. A murine MDS model had enabled demonstration of activation of β-catenin pathway leading to disease progression. This study is proof of concept that MDS therapy can target BM-MSCs and the BMM. The findings of the above two genetic studies[8,23] correlate well with previously mentioned phenotypic findings of 5-Aza treated MSCs[13,14].

Chen et al[24] in their seminal work, had performed transcriptome sequencing of BM-MSCs (MSC selection criteria: CD45-/7AAD-/CD235a-/CD31-/CD271+/CD105+) from low risk-MDS. They report transcriptome changes related to negative regulation of haematopoiesis and aberrant inflammatory signaling, thus linking MSCs to MDS pathogenesis and novel therapeutic targets. Gene expression analysis had been done by Santamaría et al[25] on de novo untreated MDS patients (N=33; MDS-WHO subtypes: 5q-, RA, RARS, RCMD, RAEB, MDS-u and hypocellular MDS) and healthy-donor-MSCs. They had reported reduced expression of miRNA in *Dicer-1*, DROSHA (gene associated with miRNA biology) and SBDS gene. Thus, this study helps to validate the findings of previous research on *Dicer-1*[9]. Further comprehensive miRNA analysis by Ozdogan et al[26] on MDS-BM-MSCs (n=10), AML-BM-MSCs (n=12) and normal-control-MSCs (n=8), had reported altered miRNA profiles for MDS-MSCs, with greater loss of expression of *Dicer-1* in AML-MSCs compared to MDS-MSCs. Thus, *Dicer-1* targeting could halter progression to AML. Studies by another group on miRNA in MDS-BM-MSCs from IPSS LR-MDS patients (n=12, control=18) had shown changes in miRNA expression in comparison to healthy donor-control-MSCs[27]. Experimentally they had confirmed negative modulation of *Dicer-1* by miRNA (miR-26b-5p and miR-148b-3p) in MDS-MSCs.

Interactions between MDS-MSCs and HSPCs and their pathological implications

Communications occurring via extracellular vesicles (EVs) between MDS-MSCs and HSPCs could provide in-depth insights into MDS pathogenesis at stem cell level. The EVs carrying miRNA cargo are of particular importance in MDS. Experimental studies conducted by Meunier et al[27] using MDS-MSCs and healthy control-MSCs, had shown that transfer of EVs (size <200nm) obtained from low risk-MDS-MSC culture supernatants to CD45+ HSCs have caused CD45+ cell apoptosis, ROS induced DNA damage, development of genetic changes associated with cancer and over expression of miRNA (miR-486-5p) in CD45+ cells. The overexpressed miR-486-5p affect genes involved in cellular signaling and immunomodulation pathways and have effects on genes implicated in haematological malignancies such as AML (Homeobox A9/HOXA9) and 5q- MDS (Ribosomal Protein S14/RPS14). Saitoh et al[28] had studied EV-miRNA derived from MDS-BM-MSC culture supernatants derived from MDS (n=22) and AML patients having myelodysplastic changes (n=7). There have been greater down regulation of miR 101 in IPSS-revised (IPSS-R) HR-MDS and leukaemia cohorts relative to IPSS-R LR-MDS. The miR 101 via epigenetic mechanisms down regulates apoptosis and gene expression. Therefore, the gene function appears to correspond with the IPSS-R score. Furthermore, miR-101 expression has found to be correlated with the blast percentage in the study group. Thus miR-101 could be a useful prognostic marker in MDS. However, the serum EV-miR-101 level has shown poor correlation to MDS-sub groups. Meunier et al[29] have recently reviewed the studies on EV and miRNA derived from MDS stroma and their clinical relevance.

Future directions

Research studies in MDS-MSCs and their progenitors have given insights into alterations occurring in MDS and their effects on the HSPCs and BMM. However, more studies are needed to validate these findings as well as decipher the exact role of MDS-MSCs in MDS pathogenesis and identify biomarkers to improve MDS outcomes of MDS[30]. Further studies into the role of EVs and miRNA in MDS could enable greater understanding of the pathogenic basis of MDS via MSC modulation of HSPCs as well as lead to development of novel non-invasive diagnostic and curative therapeutic strategies for MDS in future[29].

Conclusion

Although some studies have not detected changes in MDS-MSCs or are inconclusive, discovering definitive defects associated with MSC differentiation, immunomodulation, HSC supportive functions, cell senescence and genomic changes provide evidence for a pathogenic role of MSCs in MDS, while providing leads to biomarker discovery. Some of the identified probable MDS biomarkers include *Dicer-1* defects, MSC methylation signatures and CD271+ expression in BM-MSCs. Discovery of current MDS therapy as having effects on MSCs encourage development of BMM targeted therapies. It is widely accepted that more research is required in the field of genomics in MDS-MSCs. Initial EV derived miRNA studies have shown promising results and could provide greater understanding of MDS pathobiology and help to develop better therapies for MDS in the future.

Author contribution

Hemali W Goonasekera conceptualized and wrote the manuscript.

No relevant conflicts of interest to declare.

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