Global proteomics analysis of bone marrow: establishing TLN1 and CEP55 as potential markers for myelodysplastic syndrome

(TLN1 and CEP55 expression in myelodysplastic syndromes)

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Key words: myelodysplastic syndrome, TLN1, CEP55, bone marrow, proteomics, karyotype

Abstract

Myelodysplastic syndrome (MDS) is a hematological disorder characterized by abnormal stem cell differentiation and high risk of acute myeloid leukemia transformation. Treatment options for MDS are still limited, making the identification of molecular signatures for MDS progression a vital task. Thus, we evaluated the proteome of bone marrow plasma from patients (n = 28) diagnosed with MDS with ring sideroblasts (MDS-RS) and MDS with blasts in the bone marrow (MDS-EB), using label-free mass spectrometry. This strategy allowed the identification of 1,194 proteins in the bone marrow plasma samples. Polyubiquitin-C (UBC), Moesin (MSN) and Talin-1 (TLN1) showed the highest abundances in MDS-EB and Centrosomal protein of 55 kDa (CEP55), the highest relative abundance in the bone marrow plasma of MDS-RS patients. In a follow up, validation study, expressions of *UBC*, *MSN*, *TLN1* and *CEP55* genes were evaluated in bone marrow mononuclear cells from 45 patients, using qPCR. This second cohort included only seven patients of the first study. *CEP55, MSN* and *UBC* expressions were similar in mononuclear cells from MDS-RS and MDS-EB individuals. However, *TLN1* gene expression was greater in mononuclear cells from MDS-RS ($p = 0.049$) as compared to MDS-EB patients. Irrespective of MDS subtype, *CEP55* expression was higher ($p = 0.045$) in MDS patients with abnormal karyotypes, while *MSN, UBC* and *TALIN1* transcripts were similar in MDS with normal vs. abnormal karyotypes. In conclusion, proteomic and gene expression approaches brought evidence of altered TLN1 and CEP55 expressions in cellular and noncellular bone marrow compartments of patients with low-risk (MDS-RS) and high-risk (MDS-EB) myelodysplastic syndromes and with normal vs. abnormal karyotypes. As MDS is characterized by disrupted apoptosis and chromosomal alterations, leading to mitotic slippage, TLN1 and CEP55 represent potential markers for MDS prognosis and/or targeted therapy.

Introduction

Myelodysplastic syndromes (MDS) are hematologic stem cell malignancies associated with cytopenias, bone marrow insufficiency and anemia[1]. The pathogenesis of MDS relates to disruption of stem cell development, increased apoptosis, mutations in splicing factors and DNA repair, altered DNA methylation of tumor suppressor genes or proto-oncogenes and immune derangement[2–4]. According to the International Prognostic System (IPSS-R), MDS is grouped in very low, low, intermediate very high and high-risk disorders, depending on the pathogenesis and related alterations, and risk of acute myeloid leukemia (AML) transformation[5].

Myelodysplastic syndrome with ring sideroblasts (MDS-RS) is characterized by bone marrow dysplasia, cytopenia, ring sideroblasts ≥ 15% of bone marrow precursors, presence of *SF3B1* mutation and low-risk of AML transformation[6,7]. Ring sideroblasts are erythroid precursors with abnormal accumulation of iron inside mitochondria and MDS-RS cases have stem cells with abnormal erythroid colony formation with failure of terminal erythroid differentiation (TED)[8]. TED was also reported in high-risk MDS[8], characterized by excess blasts (MDS-EB), 5-19% myeloblasts in the bone marrow, with up to 40% of all MDS cases and a high-risk of AML transformation[6,9,10]. Low-risk MDS, such as myelodysplastic syndrome with ring sideroblasts, commonly presents symptoms of anemia and proper response to erythropoietin treatment[8]. MDS with excess blasts is linked to high-risk karyotypes such as monosomy seven, complex karyotype and mutations of genes that control disturbance of apoptosis (*TP53*) and DNA repair, stem cell maturation (*RUNX1*) and histone modifications (*ASXL*)[11].

The bone marrow contains cellular and non-cellular components that modulate the functional status of hematopoietic stem cells[12], cell-cell and cell-extracellular matrix interactions, and intracellular signaling[13]. Alterations in the biochemistry and synthesis of bone

marrow factors interfere with hematopoiesis and potentially trigger aberrant mechanisms that define MDS pathobiology. In fact, pronounced differences occur in the proteome of bone marrow plasma of patients with AML when to healthy ones[14], with upregulated and downregulated proteins associated with chemokine and cytokine signaling pathways. Protein profiles are also altered in blood and bone marrow plasma of patients with low and high-risk lymphocytic leukemia (Braoudaki et al.[15]). The proteome of bone marrow cellular compartments and blood plasma were shifted in patients with AML[16] and plasma from patients diagnosed with MDS had decreased levels of CXC chemokine ligands 4 and 7[17]. Altered plasma proteins have been detected in MDS patients with refractory anemia and with ringed sideroblasts[18] and excess blasts[19]. Other studies have also described protein profiles of platelets[20] and peripheral mononuclear cells[21] of patients with MDS. These results indicate that hematological stem cell malignancies are preceded by and/or cause dramatic alterations in the proteome of diverse biological entities, from blood to bone marrow components.

Researchers have remarkably contributed to the understanding of myelodysplastic syndrome pathophysiology[4,22–24]. Despite this unprecedented effort, the current therapy with hypomethylating agents is not effective for all cases of the disease, especially for high-risk MDS[22,25] and the identification of molecular markers for MDS progression and response to treatment is crucial for effective therapeutic strategies. Thus, a series of studies were designed to decipher potential molecular signatures of MDS subtypes. First, we evaluated the proteome of bone marrow plasma from patients diagnosed a with low-risk (MDS-RS) and a high-risk myelodysplastic syndrome (MDS-EB). As centrosomal protein of 55 kDa (CEP55), moesin (MSN), talin-1 (TLN1) and ubiquitin C (UBC) showed the highest differential abundances in MDS-RS vs. MDS-EB cases, a second phase of the research, was carried out to analyze the expression of those respective genes in mononuclear cells of a distinct cohort of MDS patients.

Materials and methods

Research design and ethical statement

The present research consisted of two independent studies (Figure 1). Study 1 evaluated the proteomes of bone marrow plasma from patients (n = 28) with a low-risk (MDS-RS) and a high-risk MDS prototype (MDS-EB). As CEP55, MSN, TLN1 and UBC proteins had the highest differential abundances in MDS-RS vs. MDS-EB cases, Study 2 was conducted to examine the expression of those genes in bone marrow mononuclear cells from an additional cohort of MDS patients (n=45).

The project was approved by the Ethics Committee of the Federal University of Ceará – UFC (#69320817.7.0000.5054), with written consent from each participant. Diagnosis of the disease followed WHO 2016 guidelines[1] and MDS risk stratification, the Revised International Prognostic Scoring System (IPSS-R)[5]. Patients were diagnosed with MDS at the Drug Research and Development Center, School of Medicine, UFC, and correspond to treatment-naive patients.

Cytogenetics

G-banding karyotype of patients from Study 1 and Study 2 (see below) was performed as previously reported[26]. Briefly, cultures were established in RPMI 1640 medium (Gibco, USA) containing 30% fetal calf serum. For the 24 h culture, colcemid was added at a 0.05 µg/mL concentration for the final 30 min. of culture. After harvesting, cells were exposed to a hypotonic KCl solution (0.068 mol/L) and fixed with Carnoy buffer (1:3 acetic acid/methanol). Slides were stained with Giemsa solution and 20 metaphases were analyzed whenever possible. The karyotype was prepared using CytoVision Automated Karyotyping System (Applied

Imaging, USA) and described according to the International System for Human Cytogenetic Nomenclature 2016[27].

Study 1. Quantitative proteomic analysis of bone marrow plasma

The proteome of bone marrow (BM) plasma was evaluated in samples from 13 patients diagnosed with MDS-RS and 15 patients with MDS-EB, which included subcategories MDS-EB-1 (5-9% blasts in the bone marrow) and MDS-EB-2 (10-19% blasts in the bone marrow) (Table 1).

Bone marrow samples were obtained by sternal aspiration at the same time as trephine biopsy, at diagnosis. After collection, BM was treated with protease inhibitors (Sigma-Aldrich, USA), centrifuged twice (700 ×*g*, 15 min.; 10,000 ×*g*, 30 min.; 4ºC) and stored at -80ºC. An aliquot of plasma was used for protein quantification[28]. BM plasma proteins (25 µg) were dissolved in buffer (8 M urea, 0.02 M TEAB, 0.5 M dithiothreitol), followed by incubation at 55°C (400 rpm agitation, 25 min). Then, iodoacetamide was added (0.014 M) and the mixture was maintained at 21°C in the dark (400 rpm, 40 min). Next, buffer (0.005 M dithiothreitol, 0.001 M $CaCl_2$ and 0.02 M TEAB) was added to reach 75µl volume. Samples were incubated with trypsin (Promega, USA) at 37°C for 18 h and trifluoroacetic acid (1%) was added to stop tryptic activity. Then, stage tip C18 columns were made using Empore TM SPE disks for peptide desalting (Sigma-Aldrich, Germany), as reported before[29,30]. Digested samples were added to the columns, washed and eluted with acetonitrile (0.5%; 25% to 80%) and 0.5% acetic acid. Peptides were quantified prior to mass spectrometry (Qubit™; Thermo Fisher, USA).

Label-free mass spectrometry

Peptides (3 µg) were applied to a Dionex Ultimate 3,000 liquid chromatographer (Thermo Scientific, USA) for reversed phase nano-chromatography[29]. Peptides were injected

into a 2 cm x 100 µm trap-column (C18, 5-µm particles), eluted to another analytical column (32 cm x 75 µm; C18, 3-µm particles; Dr. Maisch GmbH, Germany) and to the spectrometer's ionization source. Elution gradient contained formic acid and acetonitrile[29]. Samples were analyzed in positive data dependent acquisition mode (Orbitrap Elite; Thermo Fisher, USA)[29,31] and eluted fractions generated MS1 spectra (300 – 1,650 m/z, 120,000 FWHM resolution, 400 m/z). The 20 most abundant ions from MS1 with at leas two charges were selected to fragmentation (MS2) by higher-energy collisional dissociation (HCD) with an automatic gain control of 1 x 10^6 and dynamic exclusion of 10 ppm for 90 s. HCD isolation window was set for 2.0 m/z, with 5 x 10^4 AGC, normalized collision energy of 35% and threshold for fragmentation of 3,000.

Data analyses

MS1 spectra were quantified according to integrated intensity area from XIC peaks. Proteins were identified using Peaks software, with sequences from the fragmentation information and UniProt database, and protein abundances were determined with Progenesis QI (Nonlinear Dynamics; Waters, USA)[29]. A first statistical analysis (ANOVA, $p < 0.05$) was performed before protein identification to filter MS1 features. Peaks software used fragmentation spectra and *Homo sapiens* Uniprot database, considering ≤1% false discovery rate and ≥1 unique peptide per protein, in addition to other settings[29]. Proteins with different abundances ($p < 0.05$) in MDS-RS vs. MDS-EB were subjected to multivariate analysis using MetaboAnalyst 4.0 (http://www.metaboanalyst.ca)[29,32,33], setting log transformation, sample median normalization and mean centered. Partial-Least Squares Discriminant Analysis (PLS-DA) with Variable Importance in Projection (VIP) verified data patterns in the MDS-RS vs. MDS-EB scenario.

Study 2. Gene expressions in bone marrow mononuclear cells

Gene expression validation of *CEP55*, *MSN*, *TLN1* and *UBC* was carried out by qPCR in bone marrow mononuclear cells from 45 MDS patients, including 15 MDS-RS and 23 MDS-EB patients, five with multilineage dysplasia, one with single lineage dysplasia and one with MDS secondary to therapy (Table 2). Four MDS-RS and three MDS-EB patients listed in Study 1 were included in Study 2.

Bone marrow was obtained as described for Study 1. Mononuclear cells were separated after lysis of red blood cells and subjected to total RNA extraction (TRizol Reagent™; Invitrogen, USA), followed by RNA quantification (Nanodrop 2000c; Thermo Scientific, USA). cDNA was obtained with High-Capacity cDNA Reverse Transcription kits and gene expression, carried out using TaqMan Gene Expression Assays as follows: *CEP55* (Hs01070181_m1), *MSN* (Hs00792607_mH), *TLN1* (Hs00196775_m1), *UBC* (Hs00824723_m1), beta-2-microglobulin (*B2M*, Hs99999907_m1; reference gene). Real time PCR reactions were performed usgin 1x TaqMan® Universal Master Mix II, with UNG® and 7500 Fast System® (Applied Biosystems, USA). Samples were analyzed in duplicates and gene expression calculated using $2^{-\Delta Cq}$ method[37].

Statistical analysis

mRNA data distribution was evaluated by Shapiro–Wilk test and Student's t-test or one-way ANOVA with Tukey/Games Howell post hoc test was used when normality was detected. Variance homogeneity was evaluated by Levene's test and statistical analyses, conducted with SPSS 21.0 (SPSS Inc., USA) and GraphPad Prism 8 (GraphPad Prism software, USA).

***In silico* miRNA-gene networks**

Regulation of *TLN1* and *CEP55* by miRNAs was analyzed using miRNet 2.0 (https://www.mirnet.ca)[35], considering human database and bone marrow as the specific tissue. Functional enrichment of all miRNAs was built, with highlights for main biological functions.

Results

Study 1: proteome of bone marrow plasma

As determined by LC-MS/MS, 1,194 proteins were identified in the BM plasma of MDS patients, with 320 and 490 proteins unique to MDS-RS and MDS-EB samples, respectively, and 384 proteins common to both subtypes (Supplementary Table 1). Based on univariate and multivariate statistical analysis, protein patterns of MDS-RS and MDS-EB bone marrow plasma were distinct (Figure 2A), and lactoferrin, coagulation factor V, polyubiquitin-C, immunoglobulin heavy variable 3-66, inositol 1,4,5-trisphosphate receptor, moesin, histone H2B type 1-J, cDNA FLJ56274, kininogen 1 isoform, talin-1 and histone H1.5 made the most significant contributions to characterize the bone marrow plasma of MDS-EB patients. On the other hand, rheumatoid factor RF-IP12, immunoglobulin kappa var. 1-6 IgG H chain and centrosomal protein of 55 kDa defined the major representation of bone marrow plasma from MDS-RS patients (Figure 2B; Table 3). Considering fold-change values, Polyubiquitin-C (UBC), Moesin (MSN) and Talin-1 (TLN1) showed the highest relative abundances in MDS-EB and Centrosomal protein of 55 kDa (CEP55) was the major protein of the bone marrow plasma from MDS-RS patients.

Study 2. Gene expression in mononuclear cells

Expression of *CEP55, MSN* and *UBC* were similar in mononuclear cells from patients with MDS-RS and MDS-EB ($p > 0.05$). *TLN1* gene expression, however, was greater in

mononuclear cells of MDS-RS than in mononuclear cells of MDS-EB patients ($p = 0.049$). Relative quantities of *MSN, UBC* and *TLN1* transcripts were similar ($p > 0.05$) in mononuclear cells of individuals with normal and abnormal karyotypes, irrespective of being MDS subtype. However, *CEP55* gene expression was greater in MDS patients with abnormal karyotypes in comparison with patients with normal karyotypes ($p = 0.045$; Figure 3; Supplementary Table 2).

In silico miRNA-gene interactions

TLN1 expression is potentially regulated by onco-miRNAs and miRNAs associated with cell cycle, cell death, immune system, angiogenesis and hematopoiesis, among others. *CEP55*, in turn, appeared regulated by onco-miRNAs and miRNAs linked to bone regeneration, DNA damage response, cell cycle, Akt pathway, among others (Figure 4; Supplementary Table 3 and 4).

Discussion

Myelodysplastic syndromes are clinically heterogeneous and characterized, but limited to, by dysplasia of myeloid cell lines, peripheral blood cytopenia, bone marrow insufficiency, disrupted hematopoiesis and anemia[1,2–4]. Molecular determinants of MDS pathobiology are complex as well and involve altered genomic and epigenetic factors, protein sysnthesis and structure, intracellular signaling and mechanisms of cell communication, among many other aspects that determine cell life and fate. Given this scenario, our current studies were designed to advance our knowledge about the molecular landscape of MDS.

Considering the scenario with 1,194 proteins identified in the bone marrow plasma, univariate and multivariate statistical approaches grouped 15 proteins that most defined MDS-RS and MDS-EB subtypes. Among this select group, TLN1, MSN and UBC had the greatest

relative abundances in the bone marrow plasma of MDS-EB and CEP55, in MDS-RS patients. Proteomics data gathered in Study 1 and the well-known bio tasks of CEP55, MSN, UBC and TLN1 prompted us to investigate the expression of these genes in mononuclear cells obtained from a cohort of 45 patients. In this case, *TLN1* was overexpressed in mononuclear cells from MDS-RS when compared to MDS-EB patients. TLN1 is a ubiquitous protein made of alpha-helices and post-translational modification sites (https://alphafold.ebi.ac.uk/entry/Q9Y490), with actin and integrin binding domains, affecting signaling pathways[36–38]. TLN1 acts in mechanotransduction and filopodia function[39,40] and talin dysregulation results in pathological phenotypes with changed cell spreading, migration and survival[41,42]. At the molecular level, talin is associated with proliferation and migration pathways, confers anoikis resistance and metastasis[42,43], is a key player in hematologic disorders[39] and helps platelet and neutrophil activation[44]. Talin-integrin interactions affect migration of platelets and neutrophils[44,45], leukocyte and platelet function[46] and endothelial cell function, as *TLN1* knockout mouse embryos have defective angiogenesis[47]. Given MDS characteristic neutropenia and low platelet count and that sepsis is a common cause of death among MDS patients, we believe TLN1´s role in stem cell differentiation and dysplasia must be evaluated in future studies.

The reason why, in our current research, TLN1 was more abundant in MDS-EB bone marrow plasma and *TLN1* expression was prevalent in mononuclear cells of MDS-RS patients is not clearly understood. Bone marrow plasma obviously contains molecules originated from blood and it may not entirely reflect what comes from mononuclear cells. Also, mRNA and protein quantities in a given biological entity can be unrelated[48–51] and cell lysis and/or apoptosis may have occurred during collection and separation of BM plasma samples. Another possible explanation is that MDS-RS cases present serious problems related to splice machinery (mutation of *SF3B1*), leading to disrupted mRNA translation. This may have led to increased mRNA level without proportional changes in protein synthesis. In this regard, comparisons

between protein abundances in BM plasma and transcript amounts in mononuclear cells are not straightforward because two levels of molecular information were actually generated in our studies, i.e., proteomics of bone marrow plasma and transcript quantification in mononuclear cells. Thus, these two technical approaches provide complementary and not conflicting information. High TLN1 abundance in the BM plasma of patients with MDS-EB may relate to TLN1-mediated activation of FAK/AKT signaling and anoikis resistance[52]. Low *TLN1* gene expression specifically detected in mononuclear cells of MDS-EB patients could be the result of impaired talin/integrin complex.

Onco-miRNAs and miRNAs linked to cell cycle control, immune system, angiogenesis and hematopoiesis regulate *TLN1* expression, indicates our *in silico* assays. Interestingly, nine of these miRNAs have been cited by a recent publication as having differential expressions in cases of MDS[53]. Given its functional properties, TLN1 has been listed as a potential drug for targeted therapy[54]. However, more investigation is needed to decipher the precise roles of TLN1 in MDS pathobiology and how drugs would tackle protein action and/or *TLN1* gene expression.

Also defined in study 1, CEP55 was more abundant in the bone marrow plasma of MDS-RS, a distinct entity with mutations in genes related to splicing machinery. Remarkably, we observed in Study 2 that increased *CEP55* expression in mononuclear cells was linked to chromosomal abnormality in the MDS patients, regardless of MDS subtypes. CEP55 is an alpha helical protein (https://alphafold.ebi.ac.uk/entry/Q53EZ4) with domains involved in abscission, midbody localization and a ubiquitin-binding domain required for cytokinesis[55–57]. CEP55 helps to define aneuploid cells during perturbed mitosis[58] and high CEP55 expression facilitates exit from mitotic arrest, resulting in resistance to anti-mitotic drugs in murine models. CEP55-depleted organisms have increased proportion of multinucleated cells and/or cells arrested at the midbody stage[59] and, additionally, CPE55 modulates PI3K/AKT pathway[60]. In fact, based on *in silico* models (https://string-db.org/; data not shown), CEP55 interacts with a diverse array

of proteins, including components of the ESCRT-I complex, a regulator of vesicular transport, cell growth and differentiation, a microtubule-associated protein, a regulator of Rac GTPase activity and cell cycle during carcinogenesis, cyclin-dependent kinases and a DNA topoisomerase. This entire scenario reflects, thus, the multidimensional attributes of CEP55. As we have shown for the case of *TLN1*, *in silico* analysis shows *CEP55* being regulated by oncogenes and miRNAs linked to DNA damage response, cell cycle and AKT pathway. The expression of several genes that define normal blood cells are regulated by miRNAs and MDS prognosis has been linked to specific miRNAs of the bone marrow, peripheral blood and mononuclear cells[53]. In fact, some *CEP55*-controling miRNAs identified in our *in silico* model are listed by the literature as differentially expressed in patients with MDS[61–64].

Chromosomal abnormalities are common alteration observed in up to 50% of MDS cases, mostly due to problems during spindle assembly checkpoint (SAC), which controls mitotic progression and proper chromosome alignment and segregation. SAC delays anaphase until accurate kinetochore-microtubule attachment of each chromosome and inhibition of SAC prolongs mitotic arrest. The majority of antimitotic chemotherapies act by altering microtubule dynamics, but cancer cells are able to bypass mitotic arrest and prematurely exit mitosis, a phenomenon reported as mitotic slippage. High *CEP55* expression is a marker of chromosomal alterations, aneuploidy in many types of tumor and poor prognosis of cancer patients[58,65,66]. Overexpression of *CEP55* promotes tumorigenesis, causing chromosomal instability and aberrant mitotic division in a transgenic mouse model[67]. Also, transgenic mice with upregulated CEP55 have disrupted self-renewal and mitosis of spermatogonial cells in such a way that animals present a Sertoli-cell only syndrome phenotype[68]. These results support CEP55 roles in cell differentiation and cell cycle homeostasis. Chromosomal and/or genomic instability is a hallmark of cancer that leads to numerical and structural alterations in karyotypes and tumor development[69]. Cytogenetic abnormalities are the main characteristic of MDS diagnosis and

prognosis, predicting clinical evolution and transformation to AML[70,71]. Given CEP55 well-established role in cytokinesis and maintenance of aneuploidy cells, greater *CEP55* expression in patients with chromosomal alterations may relate to cytopenia, dysplasia and aberrant myeloid differentiation. CEP55 dictates the process of mitotic slippage in cancer, a process in which cancer cells exit mitosis without proper chromosome segregation, leading to chromosomal abnormalities. Considering that incidence of chromosomal abnormalities is 50% in primary MDS and up to 80% in secondary MDS, our present results are indeed exciting as CEP55 may become a target for treatment of cancer cells with cytogenetic abnormalities[72].

In conclusion, proteomic and transcriptomic approaches provided new pieces of information about molecular signatures of myelodysplastic syndromes. We recognize that our study did not evaluate other MDS subtypes such as MDS with multilineage dysplasia, MDS secondary to therapy and hypoplastic MDS. However, this is the first report of altered TLN1 and CEP55 expressions in cellular and noncellular compartments of bone marrow from patients with low and high-risk MDS and with normal vs. abnormal karyotypes. We suggest that TLN1and CEP55 could become important molecular markers for MDS prognosis and/or targeted therapy.

Acknowledgements

The authors appreciate the support from the members of the Experimental Oncology Laboratory, specially to Silvana França, from the Center for Research and Drug Development (NPDM) of the Federal University of Ceará, and from the Laboratory of Protein Chemistry and Biochemistry, at the University of Brasília.

Financial support

The present study was funded by the Ceará State Foundation for the Support of Scientific Development (FUNCAP) and The Brazilian Council for Science and Technology Development – CNPq (grant # PR2-0101-00049.01.00/15). Additional support was provided by CNPq to Cristiana L. M. Furtado (grant # 437037/2018-5), Claudia Pessoa (grants # 440755/2018-2, 434821/2018-7 and 303102/2013-6) and Arlindo A. Moura (grant # 313160/2017-1). Authors also appreciate the support of FUNCAP, CNPq and The Brazilian Commission for Higher Education (CAPES) for graduate student and post-doctoral scholarships.

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Figure 1. Overall design and main results of the research about the proteomic analysis of bone marrow plasma and gene expression in mononuclear cells from patients diagnosed with myelodysplastic syndromes (MDS). The main template of the figure was created using Biorender platform (https://biorender.com/). MDS-RS: myelodysplastic syndrome with ring sideroblasts; MDS-EB: myelodysplastic syndrome with excess blasts.

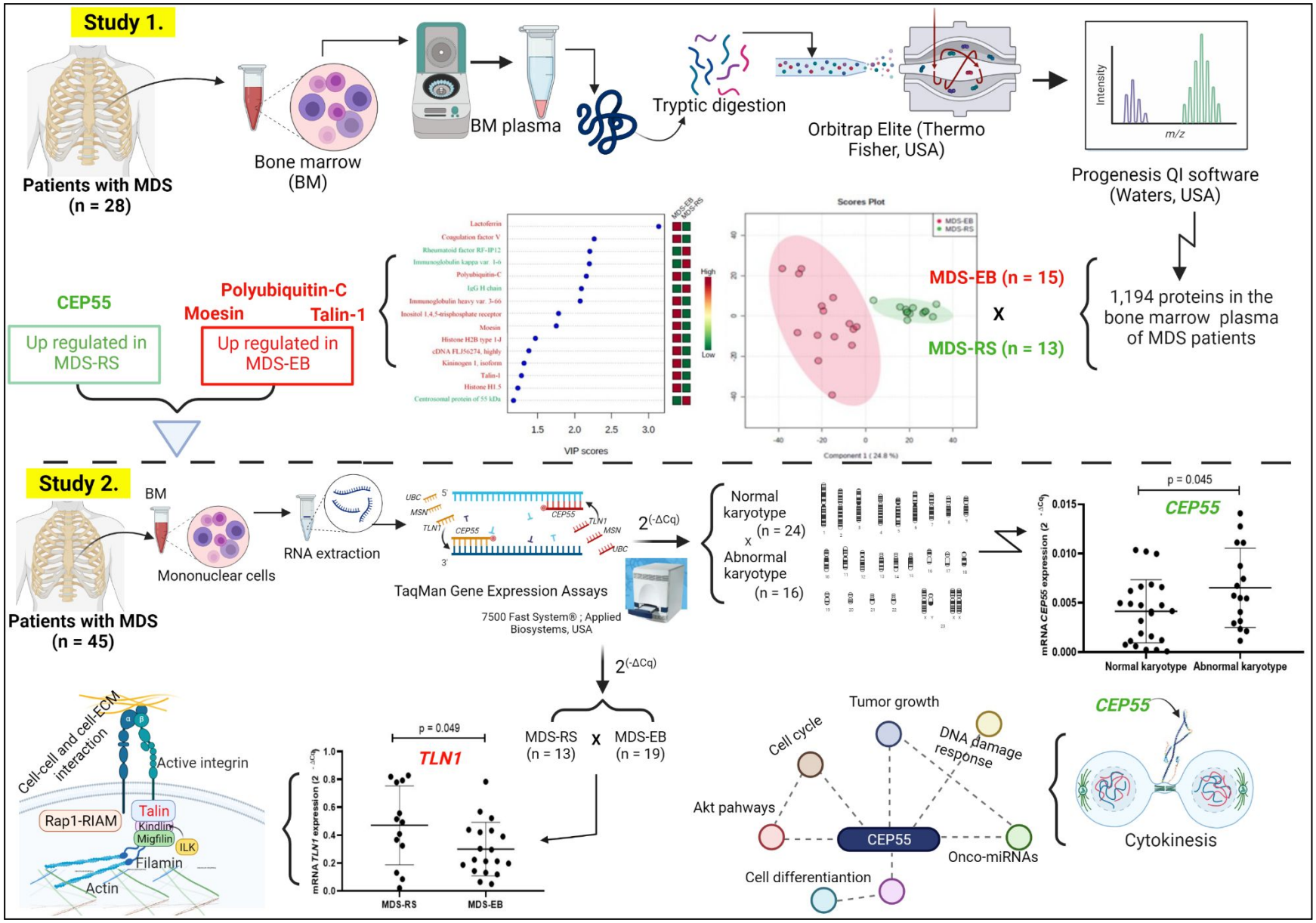
Study 1.
Patients with MDS
(n = 28)
Bone marrow
(BM)
BM plasma
Tryptic digestion
Orbitrap Elite (Thermo
Fisher, USA)
Intensity
m/z
Progenesis QI software
(Waters, USA)
1,194 proteins in the
bone marrow plasma
of MDS patients
CEP55
Up regulated in
MDS-RS
Polyubiquitin-C
Moesin
Talin-1
Up regulated in
MDS-EB
Lactoferrin
Coagulation factor V
Rheumatoid factor RF-IP12
Immunoglobulin kappa var. 1-6
Polyubiquitin-C
IgG H chain
Immunoglobulin heavy var. 3-66
Inositol 1,4,5-trisphosphate receptor
Moesin
Histone H2B type 1-J
cDNA FLJ56274, highly
Kininogen 1, isoform
Talin-1
Histone H1.5
Centrosomal protein of 55 kDa
High
Low
1.5
2.0
2.5
3.0
VIP scores
Scores Plot
MDS-EB
MDS-RS
MDS-EB (n = 15)
X
MDS-RS (n = 13)
Component 1 (24.8 %)
Study 2.
BM
Mononuclear cells
RNA extraction
Patients with MDS
(n = 45)
UBC
MSN
TLN1
CEP55
TLN1
MSN
UBC
TaqMan Gene Expression Assays
7500 Fast System® ; Applied
Biosystems, USA
2(-ΔCq)
Normal
karyotype
(n = 24)
x
Abnormal
karyotype
(n = 16)
p = 0.045
CEP55
mRNA CEP55 expression (2 -ΔCq)
0.015
0.010
0.005
0.000
Normal karyotype
Abnormal karyotype
2(-ΔCq)
MDS-RS
(n = 13)
x
MDS-EB
(n = 19)
p = 0.049
TLN1
mRNA TLN1 expression (2 -ΔCq)
1.0
0.8
0.6
0.4
0.2
0.0
MDS-RS
MDS-EB
Cell-cell and cell-ECM
interaction
Active integrin
Rap1-RIAM
Talin
Kindlin
Migfilin
ILK
Filamin
Actin
Tumor growth
Cell cycle
DNA damage
response
Akt pahways
CEP55
Onco-miRNAs
Cell differentiantion
CEP55
Cytokinesis

Table 1. Summary of clinical and prognostic characteristics of patients with myelodysplastic syndrome (MDS) with ring sideroblasts (MDS-RS) and MDS with excess blasts (MDS-EB).

Case	Gender	Age	Hb (g/dL)	ANC (/mm³)	Platelets (/mm³)	Blasts (%)	Karyotype	WHO 2016	IPSS-R
1	M	76	9	1.70	184.00	0	45,X.-Y[18]/46.XY[7]	MDS-RS	Very Low
2	F	72	4.4	1.339	101.00	1	No metaphase	MDS-RS	-
3	M	85	6.7	2.209	171.00	1	46,XY[15]	MDS-RS	Low
4	F	66	6.5	2.293	262.00	4	No metaphase	MDS-RS	-
5	F	75	8.7	3.391	382.00	1	46,XX[15]	MDS-RS	Low
6	M	60	6.12	3.643	51.83	2	No metaphase	MDS-RS	-
7	F	73	7.5	3.844	47.210	1	No metaphase	MDS-RS	-
8	F	82	5.87	2.180	267.00	2	46,XX[5]	MDS-RS	Low
9	F	70	8.07	2.294	499.00	0	No metaphase	MDS-RS	-
10	F	73	9.76	5.106	676.00	0	46,XX[12]	MDS-RS	Low
11	M	82	7.63	3.599	338.00	0	46,XX[20]	MDS-RS	Low
12	M	85	7.5	2.726	334.10	0.5	No metaphase	MDS-RS	-
13	F	87	10.9	2.728	292.00	1	No metaphase	MDS-RS	-
14	F	60	9.2	217	116.00	5	No metaphase	MDS-EB	-
15	M	59	7.7	222	31.50	5	No metaphase	MDS-EB	-
16	F	75	11	1.548	124.00	5	No metaphase	MDS-EB	-
17	M	72	7.2	813	47.00	5	No metaphase	MDS-EB	-
18	M	84	9.1	344	33.00	18	No metaphase	MDS-EB	-
19	M	58	7.2	275	22.00	18	47,XY,+8[6]/47,XY,del(7)(q32),+8[7]/46,XY[2]	MDS-EB	Very High
20	M	63	7.5	169	51.00	9	46,XY[4]	MDS-EB	High
21	M	79	7.2	1.045	87.00	19	No metaphase	MDS-EB	-
22	F	57	6	1,028	460.00	5	46,XX[10]	MDS-EB	High
23	F	86	6.7	1,280	148.00	19	46,XX[8]	MDS-EB	High
24	M	73	7.1	460	26.00	12	No metaphase	MDS-EB	-
25	M	80	11	938	128.00	8	47,XY,+8[12]/46,XY[8]	MDS-EB	Intermediate
26	M	68	5.61	289	24.00	17	46,XY[5]	MDS-EB	Very High
27	F	44	6.76	1,887	9.98	15	46,XX[20]	MDS-EB	Very High

28	M	74	12.7	843	98.00	10	46,XY,del(17)(p11.2)[3]/47,XY,+mar[3]/47,XY,+20[4]/46,XY,del(17)(p11.2),+mar[2]/46,XY[9]	MDS-EB	High

Abbreviations: ANC. Absolut Neutrophil Count; F. Female; Hb. Hemoglobin; IPSS-R. Revised International Prognostic Score System; M. Male.

Table 2. Summary of clinical and prognostic characteristics of patients with myelodysplastic syndrome (MDS).

Case	Gender	Age	Hb (g/dL)	ANC (/mm³)	Platelets (/mm³)	Karyotype	WHO 2016	IPSS-R
1	F	70	8.07	2294	449000	NO METAPHASE	MDS-RS	-
2	M	62	7.20	275	17000	47,XY,+8[6]/47,XY,del(7)(q32),+8[7]/46,XY[2]	MDS-EB	HIGH
3	M	82	7.63	3599	338000	46,XX[20]	MDS-RS	LOW
4	M	68	5.61	289	24000	NO METAPHASE	MDS-EB	-
5	F	82	5.87	2180	267000	46,XX[5]	MDS-RS	VERY LOW
6	M	74	8.60	3981	177600	46,XY[6]	MDS-RS	LOW
7	F	44	6.76	1887	9982	46,XX[20]	MDS-EB	HIGH
8	M	73	7.10	460	26000	NO METAPHASE	MDS-EB	-
9	F	76	8.70	3391	382000	46,XX[15]	MDS-RS	LOW
10	M	89	7.10	1600	104000	46,XY,t[5;19)(q13.2;q13.4)[3]/46,XY,t[5;19)(q13.2;q13.4),t(8,21)(q21.3;q22.12)[3]/46,XY,del(X)(q21),t(5;19)(q13.2;q13.4),t(8;21)(q21.3;q22.12)[5]/46,XY[9]	MDS-EB	VERY HIGH
11	M	58	7.80	2300	362000	46,XY,del(5)(q32)[3]/46,XY,del(5)(q32),del(7)(q36)[3]/46,XY,-5,+mar[9]/46,XY[7]	MDS-EB	HIGH
12	M	55	4.60	496	81000	45,XY,-7[15]/46,XY,-7,+mar[5]	MDS-EB	HIGH
13	F	42	10.50	2072	25000	46,XX[20]	MDS-EB	HIGH
14	M	84	3.90	2940	68000	46,XY[20]	MDS-EB	HIGH
15	F	79	9.90	1296	30000	46,XX[12]	MDS-EB	HIGH
16	M	75	8.30	957	21000	92, XXYY[4]	MDS-EB	HIGH
17	M	55	6.10	4460	40000	45,X,-Y[15]/45,X,-Y,del(5)(q32)[3]/46,XY[2]	MDS-EB	HIGH
18	F	81	11.90	744	57000	46,XY[4]	MDS-EB	HIGH
19	F	93	9.50	860	47000	46,XX,+8[12]/46,XX[8]	MDS-EB	HIGH
20	F	80	8.80	5461	21000	46,XX[20]	MDS-RS	LOW
21	F	77	12.00	1099	143000	46,XX[20]	MDS-EB	HIGH
22	F	82	6.90	1585	193000	47,XX,+8[9]/47,XX,+8,del(20)(q12)[5]/46,XX[6]	MDS-RS	INTERMEDIATE
23	M	79	9.90	7400	169000	46,XY[20]	MDS-EB	INTERMEDIATE
24	F	42	10.80	2079	147000	NO METAPHASE	MDS-RS	-
25	M	91	7.70	4753	203000	46,X-Y[4]/46,XY[16]	MDS-RS	LOW
26	M	58	8.50	494	300000	46,XY,del(5)(?q15q33)[8]/46,XY[12]	MDS-RS	LOW
27	M	79	6.70	4752	16000	NO METAPHASE	MDS-EB	-

28	F	72	6.50	3619	18040	NO METAPHASE	MDS-EB	-
29	F	83	9.60	1870	82000	46,XX[20]	MDS-EB	HIGH
30	F	59	9.60	3080	326000	46,XX[10]	MDS-RS	LOW
31	M	60	6.12	3643	51830	47,XY,+15[10]/46,XY[10]	MDS-RS	LOW
32	F	44	6.76	1887	9982	46,XX[20]	MDS-EB	HIGH
33	F	46	4.80	2767	556100	46,XX[20]	MDS-MLD	LOW
34	F	70	6.80	1357	23000	48,XX,del(9)(q22),r(10)(p15q26.3),+16,+18[15]/46,XX[5]	t-MDS	HIGH
35	F	56	5.2	864	28000	46,XX[20]	MDS-EB	HIGH
36	M	52	7.0	3203	6000	46,XY,del(7)(q31)[3]/45,XY,-5,del(7)(q31)[5]/46,XY,del(5)(q15),del(7)(q31)[8]46,XY,del(7)(q31),add(11)(q24)[4]/46,XY,del(5)(q15),del(7)(q31),add(11)(q24)/46,XY[5]	MDS-EB	HIGH
37	F	76	9.30	4120	234000	46,XX[20]	MDS-RS	HIGH
38	F	64	10.90	1232	20700	46 XX [10]	MDS-MLD	LOW
39	M	70	10.20	947	117400	46,XY[20]	MDS-RS	LOW
40	F	48	11.60	751	57000	46,XX[4]	MDS-SLD	LOW
41	F	76	9.90	308	90590	47,XX,+22[4]/46,XX[16]	MDS-MLD	LOW
42	M	44	15.80	852	218000	46,XY,del(5)(q15q33), del(17)(p11.2)[7]/46,XY[13]	MDS-MLD	LOW
43	F	47	8.60	2920	30000	47,XX,+6[3]/46,XX[17]	MDS-MLD	LOW
44	M	71	5.10	813	202000	46,XY[20]	MDS-EB	HIGH
45	M	92	9.20	2204	182400	46,XY,del(5)(q15q33)[7]/46,XY[13]	MDS-RS	LOW

Abbreviations: ANC. Absolut Neutrophil Count; F. Female; Hb. Hemoglobin; IPSS-R. Revised International Prognostic Score System; M. Male; MDS-EB. MDS with excess blasts (n = 23); MDS-MLD. MDS with multilineage dysplasia (n = 5); MDS-SLD. MDS with single lineage dysplasia (n = 1); t-MDS: MDS secondary to therapy (n = 1); MDS-RS. MDS with ring sideroblasts (n = 15).

Figure 2. (A) Partial least square discriminant analysis (PLS-DA) of protein abundances in the bone marrow plasma of patients with myelodysplastic syndrome with ring sideroblasts (MDS-RS) and with excess blasts (MDS-EB). Explained variances of components are shown in brackets. (B) Variable Importance in Projection (VIP) scores associated with bone marrow plasma proteins, as identified by PLS-DA. Colored boxes on the right indicate the relative abundances of proteins in each MDS subtype.

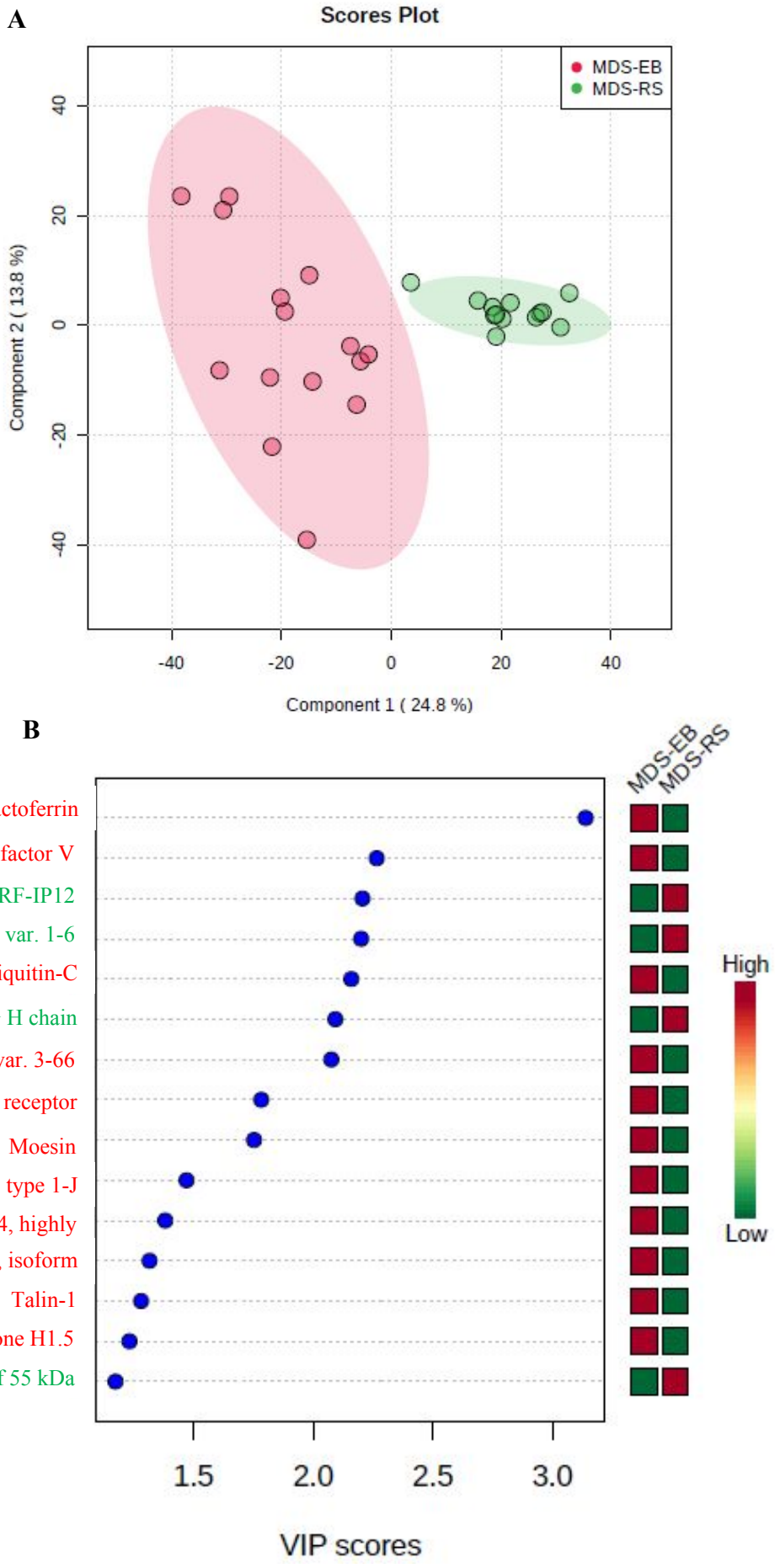
A
Scores Plot
MDS-EB
MDS-RS
Component 2 (13.8 %)
Component 1 (24.8 %)
B
Lactoferrin
Coagulation factor V
Rheumatoid factor RF-IP12
Immunoglobulin kappa var. 1-6
Polyubiquitin-C
IgG H chain
Immunoglobulin heavy var. 3-66
Inositol 1,4,5-trisphosphate receptor
Moesin
Histone H2B type 1-J
cDNA FLJ56274, highly
Kininogen 1, isoform
Talin-1
Histone H1.5
Centrosomal protein of 55 kDa
MDS-EB
MDS-RS
High
Low
VIP scores

Table 3. Proteins of the bone marrow plasma with the highest VIP scores and differentially expressed in patients with myelodysplastic syndrome with ring sideroblasts (MDS-RS) and patients diagnosed with myelodysplastic syndrome with excess blasts (MDS-EB). Proteins were identified by label-free mass spectrometry and data, analyzed by Progenesis QI software and UniProt database.

Accession number	Protein description	Higher abundance in:	Max. fold change	Confidence score*	Anova (p)
Q53EZ4	Centrosomal protein of 55 kDa GN=CEP55	MDS-RS	Infinity	24.72	0.003314
P26038	Moesin GN=MSN	MDS-EB	2713.22	71.69	0.000174
F5GZ39	Polyubiquitin-C (Fragment) GN=UB	MDS-EB	2219.84	46.85	0.004344
Q9Y490	Talin-1 GN=TLN	MDS-EB	294.77	268.42	0.022615
P06899	Histone H2B type 1-J GN=HIST1H2B	MDS-EB	138.86	47.59	0.013181
P16401	Histone H1.5 GN=HIST1H1B	MDS-EB	135.53	90.01	0.009784
P12259	Coagulation factor V GN=F	MDS-EB	104.46	25.06	0.020701
W8QEY1	Lactoferrin GN=FTF	MDS-EB	68.65	59.03	0.002933
B4E022	cDNA FLJ56274. highly similar to Transketolase (EC 2.2.1.1)	MDS-EB	68.38	203.17	0.001416
Q59ES2	Inositol 1,4,5-trisphosphate receptor type 3 variant (Fragment)	MDS-EB	56,65	30,24	0,008421
A2J1M8	Rheumatoid factor RF-IP12 (Fragment)	MDS-RS	25.93	40.95	4.88E-08
S6AWF0	IgG H chain	MDS-RS	12.07	52.97	1.94E-06
B4E1C2	Kininogen 1. isoform CRA_b OS=Homo sapiens GN=KNG1 PE=2 SV=1	MDS-EB	11.23	32.27	0.009069
A0A0C4DH72	Immunoglobulin kappa variable 1-6 GN=IGKV1-6	MDS-EB	5.89	31.18	0.001043
A0A0C4DH42	Immunoglobulin heavy variable 3-66 GN=IGHV3-66	MDS-EB	2.57	27.42	0.021628

Figure 3. Quantitative data of *TLN1* and *CEP55* expression ($2^{-\Delta Cq}$) based on qPCR analysis of total RNA extracted from bone marrow mononuclear cells of patients with myelodysplastic syndrome with ring sideroblasts (MDS-RS) and with excess blasts (MDS-EB) and patients with normal and abnormal karyotypes, as listed in Table 2.

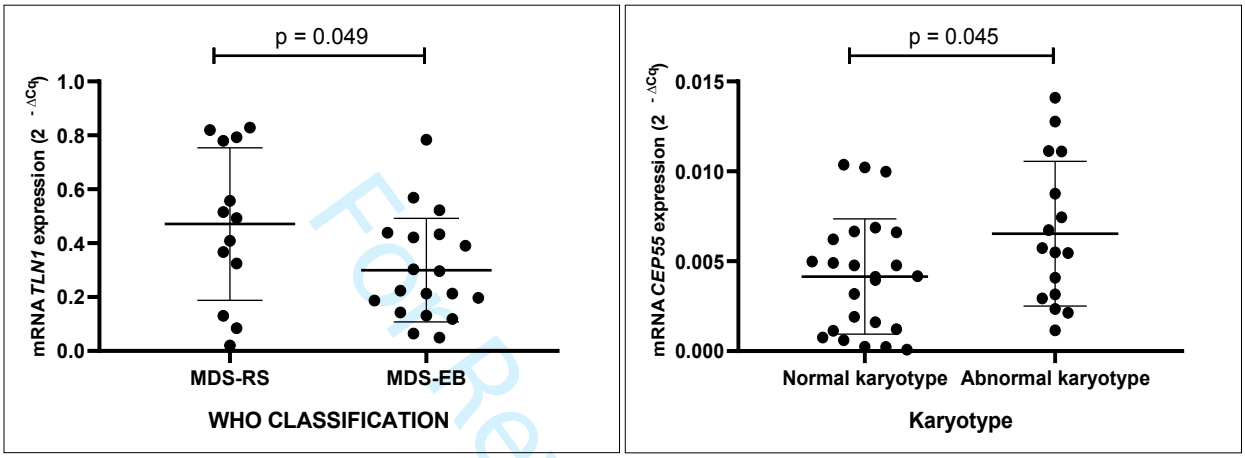

Figure 4. Network and gene set enrichment analysis of miRNAs associated with the regulation of human *TLN1* and *CEP55* based on the highest p-value obtained from miRNet database (https://www.mirnet.ca).