Peripheral Blood Mononuclear Cell Proteome Changes in Patients with Myelodysplastic Syndrome

Klara Pecankova, Pavel Majek, Jaroslav Cermak, and Jan E. Dyr
Institute of Hematology and Blood Transfusion, U Nemocnice 1, 128 20 Prague 2, Czech Republic
Correspondence should be addressed to Klara Pecankova; klara.pecankova@uhkt.cz
Received 23 September 2014; Accepted 31 March 2015
Academic Editor: Shivani Soni

Our aim was to search for proteome changes in peripheral blood mononuclear cells (PBMCs) of MDS patients with refractory cytopenia with multilineage dysplasia. PBMCs were isolated from a total of 12 blood samples using a Histopaque-1077 solution. The proteins were fractioned, separated by 2D SDS-PAGE (pI 4–7), and double-stained. The proteomes were compared and statistically processed with Progenesis SameSpots; then proteins were identified by nano-LC-MS/MS. Protein functional association and expression profiles were analyzed using the EnrichNet application and Progenesis SameSpots hierarchical clustering software, respectively. By comparing the cytosolic, membrane, and nuclear fractions of the two groups, 178 significantly ($P < 0.05$, ANOVA) differing spots were found, corresponding to 139 unique proteins. Data mining of the Reactome and KEGG databases using EnrichNet highlighted the possible involvement of the identified protein alterations in apoptosis, proteasome protein degradation, heat shock protein action, and signal transduction. Western blot analysis revealed underexpression of vinculin and advanced fragmentation of fermitin-3 in MDS patients. To the best of our knowledge, this is the first time that proteome changes have been identified in the mononuclear cells of MDS patients. Vinculin and fermitin-3, the proteins involved in cell adhesion and integrin signaling, have been shown to be dysregulated in MDS.

1. Introduction

MDS encompasses a diverse range of oncohematological diseases affecting hematopoietic stem cells and their hematopoietic microenvironment [1]. MDS is characterized by dysplastic ineffective hematopoiesis with the apoptosis of hematopoietic cells in the bone marrow and by subsequent cytopenias in the blood. It occurs in particularly elder people with incidence of 20–50 patients in 100,000 inhabitants [2]. There are several groups of MDS patients according to the WHO classification based on bone marrow and peripheral blood findings, cytogenetics, and other factors [3]. Prognostically MDS subgroups can be also stratified into low-risk and high-risk subgroups; high-risk subgroups are characterized by poor survival outcome and higher rate of progression toward acute myeloid leukemia. Refractory cytopenia with multilineage dysplasia (RCMD) is a subgroup of myelodysplastic syndrome (MDS). According to the revised WHO (World Health Organization) classification of MDS, RCMD is defined by the presence of cytopenias in peripheral blood and dysplastic changes present in 10% or more of the cells in two or more myeloid lineages in the bone marrow (with approximately 15% ringed sideroblasts) [3].

In recent years, fundamental knowledge in MDS pathophysiology based on DNA alterations has been and is still being complemented by other “omics” disciplines, in particular by proteomics, with the proteomes of plasma (of different MDS subgroups such as refractory anemia and refractory anemia with ringed sideroblasts (RA and RARS) [4], RCMD [5], refractory anemia with excess of blasts subtype 1 (RAEB-1) [6], and refractory anemia with excess of blasts subtype 2 (RAEB-2) [7]), serum [8, 9], platelets [10], and neutrophils [11] of MDS patients having been investigated. Despite research efforts the pathogenesis and exact mechanisms of MDS development still remain unclear, as there are many factors involved. For example, factors involved in the development of MDS (cytogenetic abnormalities [12], gene mutations [13–15], epigenetic alterations
Table 1: Patient characteristics.

| Patient | 1    | 2    | 3    | 4    | 5    | 6    |
|---------|------|------|------|------|------|------|
| Sex     | F    | F    | F    | F    | M    | F    |
| Age     | 70   | 33   | 69   | 59   | 73   | 65   |
| Hb [g/L]| 119  | 83   | 91   | 92   | 117  | 104  |
| RBC [10^{12}/L] | 3.15 | 2.47 | 2.33 | 2.67 | 4.29 | 2.87 |
| WBC [10^{9}/L]  | 2.20 | 2.81 | 5.50 | 4.15 | 5.10 | 2.89 |
| PLT [10^{9}/L]  | 184  | 291  | 262  | 211  | 50   | 284  |
| NS       | 1.1  | 1.91 | 3.08 | 2.41 | 2.3  | 0.72 |
| Blasts in PB [%] | 0    | 0    | 0    | 0    | 0    | 0    |
| Blasts in BM [%] | 0.4  | 0.9  | 3.2  | 4.8  | 0.8  | 3.4  |
| Karyotype | 46, XX, inv(9) | 46, XX, del(5)(q13.2q34) | 46, XX, del(5)(q31.q31) | 46, XY | 46, XX, 1qh+, 46, XX, 1qh+, del(5)(q31) |
| IPSS     | 0.5  | 0    | 0    | 0    | 0    | 0.5  |
| IPSS-R   | Low  | Low  | Low  | Low  | Very low | low |
| WPSS     | Low  | Low  | Low  | Intermediate | intermediate |
| Transfusion [avg./month] | 0.50 | 4.50 | 3.42 | 3.25 | 1.47 | 2.19 |

Hb: hemoglobin, RBC: red blood cells, PLT: platelets, NS: neutrophil segments, PB: peripheral blood, BM: bone marrow, IPSS (-R): International Prognostic Scoring System (-Revised), and WPSS: WHO Classification-Based Prognostic Scoring System.

Protein fractionation was performed using a ProteoExtract Subcellular Proteome Extraction Kit (Merck Millipore, Darmstadt, Germany) according to manufacturer instructions to enrich the proteins according to their subcellular localization; four different subproteomes were obtained (cytosolic, membrane and membrane organelle, nuclear, and cytoskeletal). Enriched proteins were precipitated with the addition of four volumes of acetone, incubated for 60 min at −20°C, and then centrifuged at 15,000 × g for 10 min. Protein concentration in all samples was determined using a Micro-BCA Protein Assay Kit (Thermo Fischer Scientific, Waltham, MA, USA). Protein sample concentrations of each subproteome were adjusted to the same level.

Isoelectric focusing was performed (IPG strips pI 4–7, 77 cm) followed by SDS-PAGE (8 × 10 cm, 10% resolving gel, 3.75% stacking gel, and 30 mA/gel), as described in detail in previous publications [4–6, 22]. Briefly, 40 μg of cytosolic, 50 μg of membrane and membrane organelle, and 40 μg of nuclear proteins were used for an IPG strip. The proteins of the cytoskeletal subproteome were not analyzed due to insufficient protein yield.

The gels were double-stained according to the improved fast-staining protocol [23], combining imidazole-zinc reverse and Coomassie dye-based staining. Imidazole-zinc reverse staining was used to detect as many spots as possible, followed by Coomassie dye-based staining to enable maximal spot detection and quantification. After each staining step, the gels were digitized and processed with Progenesis SameSpots software (Nonlinear Dynamics, Newcastle upon Tyne, UK) that computed the fold and P values of all spots using one-way ANOVA analysis. Protein spots that differed significantly (P < 0.05) were submitted for protein
identification by tandem mass spectrometry (HCT ultra ion-trap mass spectrometer with nanoelectrospray ionization; Bruker Daltonics, Bremen, Germany) coupled to a nanoLC system (UltiMate 3000; Dionex, Sunnyvale, CA, USA); this procedure has been described in detail previously [4–6, 22]. Mascot (Matrix Science, London, UK) was used for database searching (Swiss-Prot). Two unique peptides (with higher Mascot scores than the minimum for identification, $P < 0.05$) were necessary to successfully identify a protein. Exceptions were given to proteins with a molecular weight of 15 kDa or less and to proteins with more than 3 additional unique peptides identified by error tolerant search.

To analyze the functional associations between identified proteins and cellular pathways, the protein list was processed with the on-line EnrichNet application [24] using KEGG [25, 26] and Reactome [27, 28] databases. The significance of overlap between protein sets was evaluated using a combination of one-side Fisher’s exact test ($q < 0.05$) and network similarity scores (XD-scores). The threshold values were estimated via EnrichNet with a regression fit equivalent to a Fisher $q$ value of 0.05 and an upper boundary of 95% confidence for linear fitting.

Dendrogram analysis was performed using Progenesis SameSpots software to reveal closely related proteins. The dendrogram is a visual representation of spot correlation data (with correlation analysis performed on log-normalized spot expression levels). Spots were clustered according to their closest correlation.

Western blot analysis was performed for vinculin and fermitin-3 proteins. Equal protein amounts of all (patient or donor) samples of appropriate protein fractions were pooled and 1 $\mu$g or 0.75 $\mu$g of total protein amounts were used for 1D western blot analyses for vinculin or fermitin-3, respectively. Briefly, following SDS-PAGE (10% resolving gel) proteins were transferred to a PVDF membrane (10 V constant voltage for 60 min) using an Owl HEP-1 Semi Dry Electroblotting System (Thermo Scientific, Waltham, MA, USA). Membranes were then incubated with a blocking buffer (3% BSA in PBS) at 30 $^\circ$C for 60 min and incubated with primary antibodies, anti-vinculin antibody (V9131; Sigma-Aldrich, Prague, Czech Republic) (1:200 dilution) or anti-keratin-3 antibody (SAB4200013; Sigma-Aldrich, Prague, Czech Republic) (1:340 dilution). Then the membranes were incubated with secondary antibodies, rabbit anti-mouse IgG antibody conjugated with peroxidase (for vinculin detection, 1:80,000 dilution) (A9044; Sigma-Aldrich, Prague, Czech Republic) or goat anti-rabbit IgG antibody conjugated with peroxidase (for keratin-3 detection, 1:120,000 dilution) (A0545; Sigma-Aldrich, Prague, Czech Republic). Visualization was performed using a chemiluminescent substrate (SuperSignal West Pico; Thermo Scientific, Waltham, MA, USA) and CL-XPosure Film (Thermo Scientific, Waltham, MA, USA).

### 3. Results and Discussion

Comparing the PBMC subproteomes of RCMD patients ($n = 6$) with healthy volunteer control group subproteomes ($n = 6$), we found 178 spots that differed significantly in their normalized volumes ($P < 0.05$). Figures 1–3 indicate the positions of significantly differing spots of the cytosolic, membrane and membrane organelle, and nuclear subproteomes, respectively. The spots are marked considering the gel staining.

Proteins of the spots detected were submitted to protein identification by mass spectrometry, and 139 unique proteins were identified. The list of all spots, including ANOVA $P$ values, their multiplication (fold value), protein identification with the number of identified peptides (unique peptides...
PBMCs can secrete proteins into the plasma [29, 30]; cell pathways (Tables 3 and 4). XD-scores were considered to reveal implications of the identified proteins in several cell-pathogenesis, we processed the proteindataset in EnrichNet. Identified proteins in the PBMC cellular and metabolic processes are related to these fragments; similar results were observed for complement proteins. However, it should be taken into account that PBMCs are not the only source of these plasma proteins.

In order to estimate the possible involvement of the identified proteins in the PBMC cellular and metabolic pathways and thus reveal the processes influenced by RCMD pathogenesis, we processed the protein dataset in EnrichNet. Enrichment analysis using KEGG and Reactome databases revealed implications of the identified proteins in several cellular pathways (Tables 3 and 4). XD-scores were considered to be more than 0.78 and 1.0 threshold values, as estimated by the application for the KEGG and Reactome databases, respectively.

Dendrogram analysis was performed using Progenesis SameSpots, which grouped spots by their expression profiles using an automatic correlation analysis and hierarchical clustering. We chose the top ten spot expression profile groups (with distance parameters less than 0.5) with almost identical expression profiles. Thus, each group contained spots with similar expression profiles, suggesting that these spots may be coregulated, colocalized, or by another way coaffected. The list of the expression profile groups (denoted as A, B, etc.), including the spot number, protein identification, and its accession number, is presented in Tables 5–7. Proteins in the F1 groups (cytosolic subproteome) were associated with the cytoskeleton, microtubule metabolism, cellular homeostasis maintained by heat shock proteins (HSPs), and proteosome. Proteins in the F2 groups (membrane and membrane organelle subproteome) were associated with the mitochondria and apoptosis. The protein identified in most cases in the F3 groups (nuclear subproteome) was Filamin-A, which is released from the apoptotic nucleus [31]. Other proteins in these groups were mainly actin and actin-binding proteins. Thus, the actual associations corresponded to the subproteomes as anticipated. Due to thematic similarity of the protein groups obtained via EnrichNet and dendrogram analysis, we discuss the results in parallel. Further in relation to MDS or other hematological malignancies, we summarize the most interesting protein groups that could be affected in connection with pathophysiological processes.

PBMCs are metabolically active cells (carbohydrate metabolism, cellular respiration) and as cells of the immune system are involved in antigen processing and presentation. Our KEGG results analysis (Table 3) highlighted the relationships of the identified proteins to infectious agents (E. coli). This observation is not surprising, as it is known that the immune system in MDS is dysregulated and MDS patients tend to be especially vulnerable to infections [17, 32]. Therefore, this observation is most likely related to the manifestation of secondary complications, rather than MDS itself.

T-complex protein 1 (TCP-1) subunits (four of eight identified, Table 2) with a degree of functional autonomy [33] are a part of the TRiC cytosolic chaperone (TCP-1 ring complex), which acts in tubulin biosynthesis (Table 4). This chaperone was originally thought to fold only cytoskeletal proteins but now is known to have a more general role in protein folding in eukaryotic cytosol [34]. TRiC also assists in the formation of BBSome, a part of the primary cilium, nonmotile microtubule-based sensory organelle transporting signals within the cell [35]. Primary cilium has not been under closer analysis until in the last decade, and many questions surrounding it are still unanswered. For example, it is not entirely known, whether the primary cilium is present in PBMCs [36–39]. There is evidence that components which contribute to the assembly of the primary cilium are expressed by PBMCs [36]. Therefore, there is a possibility that TCP subunits are part of the machinery of primary cilium formation or function, as a chaperone not only of cytoskeletal proteins (see Table 5, Groups F1A, F1B). Changes in TCP subunits could cause changes or even errors in BBSome formation [40], thus an effect on signaling within the cell. In last decade, the function of primary cilium in several cancers has been described, but its role in hematological malignancies...
Table 2: List of spots that differed significantly when RCMD patients were compared with a control group of healthy volunteers.

| Spot | Protein | Uniprot AC       | Peptides | P       | Fold |
|------|---------|------------------|----------|---------|-------|
| 1    | Vinculin| P18206           | 5        | 0.0017  | 2.7   |
| 2    | Tubulin alpha-1C chain; tubulin alpha-1B chain | Q9BQE3; P68363 | 4        | 0.00478 | 2.6   |
|      |         | P08133           | 2        |         |       |
| 3    | Alpha-enolase | P06733         | 8        | 0.026   | -2.5  |
| 4    | Alpha-enolase | P06733         | 6        | 0.0345  | -2.4  |
| 5    | Cytosolic nonspecific dipeptidase | Q96KP4 | 14       | 0.00179 | -2.3  |
|      | Tubulin beta chain | P07437      | 5        |         |       |
| 6    | Annexin A1 | P04083           | 10       | 0.00387 | -2.4  |
| 7    | Leukotriene A-4 hydrolase | P09960   | 12       | 0.000041 | -2.1  |
| 8    | T-complex protein 1 subunit theta | P50990 | 6        | 0.00287 | -1.7  |
| 9    | Actin, cytoplasmic 1; actin, cytoplasmic 2 | P60709; P63261 | 3        | 0.0133  | 2.1   |
| 10   | Plastin-2 | P13796           | 17       | 0.006047 | -3.3  |
| 11   | Protein S100-A9 | P06702     | 1        | 0.0279  | -2.0  |
| 12   | Neutral alpha-glucosidase AB | Q14697 | 3        | 0.012   | -2.0  |
| 13   | Leukocyte elastase inhibitor | P30740 | 8        | 0.0114  | -2.0  |
| 14   | Alpha-enolase | P06733         | 4        |         |       |
|      | Adenylosuccinate synthetase isozyme 2 | P30520 | 5        | 0.000782 | -2.0  |
|      | Rab GDP dissociation inhibitor beta | P50395 | 3        |         |       |
|      | C-terminal-binding protein 1 | Q13363 | 2        |         |       |
| 15   | Annexin A7 | P20073           | 3        | 0.00019 | -2.0  |
|      | Adenosylhomocysteinase | P23526 | 3        | 0.00019 | -2.0  |
|      | Proliferation-associated protein 2G4 | Q9UQ80 | 1        |         |       |
| 16   | Actin, cytoplasmic 1; actin, cytoplasmic 2 | P60709; P63261 | 3        | 0.0247  | 1.9   |
|      | T-complex protein 1 subunit beta | P78371 | 2        |         |       |
| 17   | Vinculin | P18206           | 5        | 0.000267 | 1.9   |
| 18   | Triosephosphate isomerase | P60174 | 5        | 0.000113 | -1.7  |
| 19   | Eukaryotic initiation factor 4A-1 | P60842 | 6        | 0.000113 | -1.7  |
|      | Heterogeneous nuclear ribonucleoprotein F | P52597 | 4        | 0.000589 | -1.9  |
|      | Heterogeneous nuclear ribonucleoprotein Q | O60506 | 4        | 0.000589 | -1.9  |
|      | T-complex protein 1 subunit theta | P50990 | 3        |         |       |
| 20   | Plastin-2 | P13796           | 14       | 0.00156 | -1.9  |
| 21   | Alpha-enolase | P06733         | 7        | 0.00129 | -1.9  |
| 22   | Plastin-2 | P13796           | 16       | 0.00363 | -1.9  |
| 23   | Proteosome subunit alpha type-6 | P60900 | 4        | 0.000319 | -1.8  |
|      | UPF0568 protein C1orf166 | Q9Y224 | 4        | 0.000319 | -1.8  |
|      | Hypoxanthine-guanine phosphoribosyltransferase | P00492 | 2        |         |       |
| 24   | Alpha-enolase | P06733         | 6        | 0.0172  | 1.8   |
| 25   | Ubiquitin carboxyl-terminal hydrolase 5 | P45974 | 5        | 0.00228 | -1.7  |
| 26   | Proteosome activator complex subunit 1 | Q63232 | 2        | 0.000034 | -1.8  |
|      | Coagulation factor XIII A chain | Q00488 | 2        | 0.000034 | -1.8  |
| 27   | Heat shock cognate 71 kDa protein | P1142 | 4        | 0.0017  | 1.7   |
|      | Rho-related GTP-binding protein RhoC. | P08134 | 2        | 0.0017  | 1.7   |
| 28   | 40S ribosomal protein SA | P08865 | 7        | 0.00126 | -1.8  |
| 29   | Triosephosphate isomerase | P60174 | 7        | 0.0217  | -1.8  |
| 30   | T-complex protein 1 subunit zeta | P40227 | 4        | 0.00972 | -1.8  |
| 31   | WD repeat-containing protein 1 | O75083 | 9        | 0.0183  | -1.8  |
| Spot | Protein                                                                 | Uniprot AC   | Peptides | P        | Fold |
|------|-------------------------------------------------------------------------|--------------|----------|---------|------|
| 32   | Actin, cytoplasmic 1; actin, cytoplasmic 2                            | P60709; P63261 | 2        | 0.00152 | 1.7  |
|      | Heterogeneous nuclear ribonucleoprotein K                             | P61978       | 2        | −0.00181| −1.6 |
|      | ADP-sugar pyrophosphatase                                              | Q9UKK9       | 2        | −0.000127| −1.6 |
| 33   | Coronin-1A                                                             | P31146       | 2        | 0.00433 | −1.7 |
| 34   | 26S proteasome non-ATPase regulatory subunit 14                        | O00487       | 2        | 0.000458| −1.7 |
| 35   | m7GpppX diphosphatase                                                  | Q96C86       | 4        | 0.00316 | −1.5 |
|      | Transaldolase                                                          | P37837       | 3        | −0.0000838| −1.5 |
| 36   | Proliferation-associated protein 2G4                                    | Q9UQ80       | 3        | 0.000667| −1.7 |
|      | Annexin A1                                                             | P40483       | 2        | −0.0000838| −1.5 |
| 37   | Macrophage-capping protein                                              | P40121       | 3        | 0.0279  | −1.7 |
| 38   | Talin-1                                                                 | Q9Y490       | 5        | 0.0497  | 1.7  |
| 39   | Heat shock 70 kDa protein 4                                              | P34932       | 5        | 0.0282  | −1.7 |
| 40   | Plastin-2                                                               | P13796       | 17       | 0.0131  | −1.6 |
| 41   | Glutathione S-transferase P                                             | P09211       | 6        | 0.00628 | −1.8 |
| 42   | Protein S100-A7                                                         | P31151       | 2        | 0.00218 | −1.6 |
| 43   | Talin-1                                                                 | Q9Y490       | 4        | 0.0804  | 1.6  |
| 44   | Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A    | P30153       | 7        | 0.0245  | −1.6 |
|      | alpha isoform                                                           | P13489       | 3        | 0.0247  | −1.6 |
| 45   | Ribonuclease inhibitor                                                  | P08133       | 20       | 0.000508| −2.6 |
| 46   | Actin, cytoplasmic 1; actin, cytoplasmic 2                              | P60709; P63261| 3        | 0.00181 | 1.6  |
| 47   | Tubulin beta-1 chain                                                    | Q9H4B7       | 2        | 0.0144  | 1.6  |
| 48   | Vinculin                                                                | P18206       | 3        | 0.0104  | 1.6  |
| 49   | Annexin A5                                                             | P08758       | 12       | 0.00129 | −1.8 |
| 50   | Proteasome activator complex subunit 2                                   | Q9UL46       | 3        | 0.000338| −1.6 |
| 51   | Annexin A6                                                              | P08133       | 20       | 0.0336  | −1.5 |
|      | Heat shock 70 kDa protein 1A/IB                                          | P08107       | 3        | 0.000508| −2.6 |
|      | Heat shock 70 kDa protein 1-like                                        | P08107       | 3        | 0.000508| −2.6 |
|      | Heat shock protein HSP 90-alpha                                         | P34931       | 2        | 0.0282  | −1.7 |
|      | Heat shock protein HSP 90-alpha                                         | P07900       | 2        | 0.0282  | −1.7 |
| 52   | Actin, cytoplasmic 1; actin, cytoplamic 2                               | P60709; P63261| 2        | 0.000127| −1.6 |
|      | Glutathione S-transferase P                                             | P09211       | 3        | 0.000127| −1.6 |
| 53   | Actin, cytoplasmic 1; actin, cytoplamic 2                               | P60709; P63261| 2        | 0.0352  | 1.5  |
| 54   | Triosephosphate isomerase                                               | P60174       | 2        | 0.00181 | −1.5 |
| 55   | Ras GTPase-activating-like protein IQGAP1                                | P46940       | 13       | 0.0219  | −1.7 |
| 56   | Actin, cytoplasmic 1; Actin, cytoplamic 2                               | P60709; P63261| 2        | 0.019   | 1.5  |
| 57   | Rab GDP dissociation inhibitor beta                                      | P50395       | 3        | 0.0336  | −1.5 |
|      | 26S proteasome non-ATPase regulatory subunit II                         | O00231       | 2        | −0.0000838| −1.5 |
| 58   | Rab GDP dissociation inhibitor beta                                      | P50395       | 13       | 0.000838| −1.5 |
|      | Alpha-enolase                                                           | P06733       | 6        | 0.000838| −1.5 |
|      | Adenylosuccinate synthetase isozyme 2                                   | P30520       | 5        | −0.0000838| −1.5 |
|      | Beta-centractin                                                         | P42025       | 3        | −0.0000838| −1.5 |
| 59   | Tubulin alpha-1C chain                                                  | Q9BQE3       | 5        | 0.0393  | 1.5  |
|      | Tubulin alpha-1A chain                                                  | Q71U36       | 5        | 0.0393  | 1.5  |
|      | Tubulin alpha-4A chain                                                  | P68366       | 4        | 0.0393  | 1.5  |
| 60   | Alpha-enolase                                                           | P06733       | 7        | 0.0459  | 1.5  |
|      | Phosphoglycerate kinase 1                                               | P00558       | 8        | 0.0459  | 1.5  |
| 61   | Lymphocyte-specific protein 1                                            | P33241       | 2        | 0.00972 | −1.5 |
|      | Secernin-1                                                              | Q12765       | 3        | 0.00972 | −1.5 |
| Spot | Protein                                                                 | Uniprot AC | Peptides | P     | Fold |
|------|-------------------------------------------------------------------------|------------|----------|-------|------|
| 62   | Eukaryotic translation initiation factor 3 subunit H                    | O15372     | 3        | 0.00335 | −1.5 |
|      | T-complex protein 1 subunit eta                                        | Q99832     | 4        |        |      |
| 63   | L-Lactate dehydrogenase B chain                                         | P07195     | 8        | 0.00156 | −1.5 |
|      | Pyruvate kinase isozymes M1/M2                                          | P14618     | 5        |        |      |
| 64   | 6-Phosphogluconolactonase                                               | O95336     | 5        | 0.0029  | −1.5 |
|      | Endoplasmic reticulum resident protein 29                               | P30040     | 4        |        |      |
| 65   | Pyruvate kinase isozymes M1/M2                                          | P14618     | 8        | 0.000801 | −1.7 |
|      | Sorting nexin-6                                                         | Q9UNH7     | 2        |        |      |
| 66   | Proteasome subunit beta type-3                                           | P49720     | 3        | 0.00454 | −1.5 |
|      | Triosephosphate isomerase                                               | P60174     | 5        |        |      |
| 67   | Pyruvate kinase isozymes M1/M2                                          | P14618     | 16       | 0.0219 | −1.5 |
| 68   | 14-3-3 protein zeta/delta                                                | P63104     | 6        | 0.0053  | −1.6 |
| 69   | 4-trimethylaminobutyraldehyde dehydrogenase Caspase-1                   | P49189     | 2        | 0.0183  | −1.4 |
|      |                                                                           | P29466     | 2        |        |      |
| 70   | Proteasome subunit beta type-4                                           | P28070     | 2        | 0.000337 | −1.4 |
| 71   | Chloride intracellular channel protein 1                                 | O00299     | 6        | 0.00012 | −1.7 |
|      | Tumor protein D5                                                         | O43399     | 2        |        |      |
| 72   | Major vault protein                                                      | Q14764     | 8        | 0.0122  | −1.7 |
|      | Ubiquitin-like modifier-activating enzyme 1                              | P22314     | 8        |        |      |
| 73   | Transitional endoplasmic reticulum ATPase                                | P55072     | 13       | 0.0132  | −1.8 |
|      | Ras GTPase-activating-like protein IQGAPI                                  | P46940     | 3        |        |      |
| 74   | Serum albumin                                                           | P02768     | 10       | 0.00454 | −1.7 |
| 75   | Heat shock protein beta-1                                                | P04792     | 2        | 0.00187 | 1.5  |
| 76   | Vinculin                                                                | P18206     | 6        | 0.00886 | −1.5 |
| 77   | Gelsolin                                                                | P06396     | 7        | 0.0459  | −2.1 |
| 78   | Actin, cytoplasmic 1; actin, cytoplasmic 2                               | P60709; P63261 | 5  | 0.0221 | 1.9  |
|      | Annexin A1                                                              | P40483     | 3        |        |      |
| 79   | Tryptophan-tRNA ligase, cytoplasmic                                      | P23381     | 7        | 0.00185 | −1.6 |
| 80   | Vinculin                                                                | P18206     | 7        | 0.0369  | −1.7 |
| 81   | Proteasome activator complex subunit 1                                   | Q06323     | 7        | 0.0328  | −1.5 |
| 82   | Cofilin-1                                                                | P23528     | 3        | 0.000356 | −1.7 |
| 83   | Endoplasmic reticulum ATPase                                            | P14625     | 5        | 0.00491 | −2.6 |
| 84   | Actin, cytoplasmic 1; actin, cytoplasmic 2                               | P60733     | 4        | 0.000166 | 2.4  |
|      | Alpha-actinin-1                                                          | Q99685     | 1        |        |      |
| 85   | Alpha-actinin-1                                                          | P12814     | 2        | 0.00777 | 2.4  |
| 86   | Heterogeneous nuclear ribonucleoprotein H2                              | P55795     | 4        | 0.000792 | −2.3 |
|      | Heterogeneous nuclear ribonucleoprotein H                                | P31943     | 3        |        |      |
| 87   | Actin, cytoplasmic 1; actin, cytoplasmic 2                               | P60709; P63261 | 3  | 0.00752 | 2.3  |
|      | Alpha-actinin-1                                                          | P12814     | 2        |        |      |
| 88   | Actin, cytoplasmic 1; actin, cytoplasmic 2                               | P60709; P63261 | 3  | 0.00752 | 2.3  |
|      | Alpha-actinin-1                                                          | P12814     | 2        |        |      |
| 89   | Delta(3,5)-delta(2,4)-dienoyl-CoA isomerase, mitochondrial              | Q13011     | 3        | 0.000218 | −2.2 |
|      | Estradiol 17-beta-dehydrogenase                                          | Q92506     | 2        |        |      |
| 90   | Heterogeneous nuclear ribonucleoproteins A2/B1                           | P22626     | 3        | 0.000986 | −2.2 |
| 91   | Transitional endoplasmic reticulum ATPase                                | P55072     | 5        | 0.00163 | −2.1 |
| 92   | Endoplasmin                                                              | Q99798     | 7        | 0.000362 | −2.1 |
|      |                                                                           | P14625     | 2        |        |      |
| 93   | Endoplasmin                                                              | P14625     | 7        | 0.000319 | −2.0 |
| Spot | Protein                                                                 | Uniprot AC | Peptides | P       | Fold  |
|------|-------------------------------------------------------------------------|------------|----------|---------|-------|
| 93   | Heat shock 70 kDa protein 1A/1B                                         | P08107     | 4        |         |       |
|      | Dihydrolipoyllysine-residue acetyltransferase component of pyruvate     | P10515     | 6        | 0.00155 | −2.0  |
|      | dehydrogenase complex, mitochondrial                                   |            |          |         |       |
|      | Stress-70 protein, mitochondrial                                        | P38646     | 3        |         |       |
|      | Annexin A6                                                              | P08133     | 3        |         |       |
| 94   | Heat shock protein 75 kDa, mitochondrial                                 | Q12931     | 3        | 0.00144 | −2.0  |
| 95   | 78 kDa glucose-regulated protein                                         | P11021     | 17       |         |       |
|      | Protein disulfide-isomerase A4                                           | P13667     | 4        | 0.0018  | −2.5  |
|      | Endoplasmic reticulum resident protein 29                                | P14625     | 2        |         |       |
| 96   | Endoplasmic reticulum resident protein 29                                | P30040     | 6        | 0.00318 | −1.9  |
|      | ATP synthase subunit gamma, mitochondrial                                | P36542     | 2        |         |       |
| 97   | Endoplasmic reticulum resident protein 29                                | P14625     | 7        | 0.0109  | −1.9  |
| 98   | Tropomyosin alpha-4 chain                                                | P67936     | 4        | 0.00755 | 2.1   |
| 99   | 78 kDa glucose-regulated protein                                         | P11021     | 7        | 0.00377 | −1.8  |
| 100  | Endoplasmic reticulum resident protein 29                                | P14625     | 5        | 0.0108  | −1.8  |
| 101  | 40S ribosomal protein SA                                                 | P08865     | 3        |         |       |
|      | Vimentin                                                                | P08670     | 4        | 0.00000707 | −1.8 |
| 102  | F-actin-capping protein subunit beta                                      | P47756     | 2        | 0.000949 | −1.8  |
| 103  | 78 kDa glucose-regulated protein                                         | P11021     | 15       | 0.00946 | −2.1  |
| 104  | Superoxide dismutase [Cu-Zn]                                             | P00441     | 1        | 0.0139  | 1.7   |
|      | ATP synthase subunit alpha, mitochondrial                                | P25705     | 1        |         |       |
| 105  | Actin, cytoplasmic 1; actin, cytoplasmic 2                               | P60709; P63261 | 4        | 0.00138 | 1.7   |
| 106  | F-actin-capping protein subunit alpha-1                                   | P52907     | 2        | 0.00247 | 1.7   |
| 107  | Purine nucleoside phosphorylase                                          | P00491     | 2        | 0.00353 | −1.7  |
|      | Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 1  | P04843     | 2        |         |       |
| 108  | Stress-70 protein, mitochondrial                                         | P38646     | 10       | 0.00224 | −1.6  |
|      | Heat shock cognate 71 kDa protein                                        | P11142     | 4        |         |       |
| 109  | Stress-70 protein, mitochondrial                                         | P38646     | 2        | 0.0137  | 1.7   |
| 110  | Actin, cytoplasmic 1; actin, cytoplasmic 2                               | P60709; P63261 | 2        | 0.00201 | 1.7   |
| 111  | Delta(3,5)-(3,2,4)-dienoyl-CoA isomerase, mitochondrial                 | Q13011     | 3        | 0.00338 | −1.7  |
| 112  | 60 kDa heat shock protein, mitochondrial                                 | P10809     | 6        | 0.00385 | 1.7   |
|      | Integrin beta-3                                                          | P05106     | 4        |         |       |
|      | ATP synthase subunit beta, mitochondrial                                 | P06576     | 2        |         |       |
| 113  | Aldehyde dehydrogenase, mitochondrial                                    | P05091     | 9        | 0.000949 | −1.7  |
| 114  | Heat shock cognate 71 kDa protein                                        | P11142     | 8        |         |       |
|      | Stress-70 protein, mitochondrial                                         | P38646     | 4        | 0.0122  | −1.7  |
|      | Endoplasmic reticulum resident protein 29                                | P14625     | 4        |         |       |
| 115  | T-complex protein 1 subunit beta                                         | P78371     | 5        | 0.0215  | −1.7  |
| 116  | Moesin                                                                  | P26038     | 4        | 0.000902 | −1.6  |
|      | T-complex protein 1 subunit zeta                                         | P78371     | 2        |         |       |
| 117  | Macrophage-capping protein                                                | P40121     | 2        | 0.0114  | −1.6  |
| 118  | Stress-70 protein, mitochondrial                                         | P38646     | 13       | 0.0106  | −1.7  |
|      | Annexin A6                                                               | P08133     | 3        |         |       |
| 119  | 60 kDa heat shock protein, mitochondrial                                 | P10809     | 11       | 0.000211 | −1.6  |
|      | Heterogeneous nuclear ribonucleoprotein K                                | P61978     | 2        |         |       |
| 120  | Elongation factor Tu, mitochondrial                                      | P49411     | 4        | 0.00392 | −1.5  |
|      | Isocitrate dehydrogenase [NADP] cytoplasmic                             | O73874     | 2        |         |       |
Table 2: Continued.

| Spot | Protein                                             | Uniprot AC | Peptides | $P$     | Fold |
|------|-----------------------------------------------------|------------|----------|---------|------|
| 121  | Pre-mRNA-processing factor 19                       | Q9UMS4     | 2        | 0.00523 | −1.6 |
| 122  | Prohibitin                                          | P35232     | 9        | 0.0121  | −1.6 |
|      | Annexin A2                                          | P07355     | 6        |          |      |
| 123  | 78 kDa glucose-regulated protein                    | P11021     | 6        |          |      |
|      | ATP synthase subunit beta, mitochondrial            | P06576     | 4        | 0.0216  | −1.6 |
|      | Integrin alpha-IIb                                  | P08514     | 2        |          |      |
| 124  | DnaJ homolog subfamily B member 11                  | Q9URS4     | 2        | 0.00315 | −1.6 |
|      | Moesin                                              | P26038     | 2        |          |      |
| 125  | Protein disulfide-isomerase                          | P07237     | 7        | 0.0422  | −1.6 |
| 126  | Gelsolin                                            | P06396     | 3        | 0.0294  | 1.5  |
| 127  | 14-3-3 protein zeta/delta                            | P63104     | 5        | 0.0337  | 1.5  |
|      | 14-3-3 protein eta                                   | Q04917     | 2        |          |      |
| 128  | 60 kDa heat shock protein, mitochondrial            | P10809     | 18       | 0.00252 | −1.5 |
| 129  | Endoplasmnin                                        | P14625     | 4        | 0.0251  | −1.6 |
| 130  | Synaptosomal-associated protein 23                  | O00161     | 3        | 0.0254  | 1.5  |
| 131  | Stress-induced-phosphoprotein 1                      | P31948     | 1        | 0.0329  | −1.5 |
| 132  | Calreticulin                                        | P27797     | 2        | 0.00435 | −1.5 |
| 133  | UPF0568 protein C1orf166                             | Q9Y224     | 4        | 0.0208  | −1.5 |
| 134  | 60 kDa heat shock protein, mitochondrial            | P10809     | 7        |          |      |
|      | ATP synthase subunit beta, mitochondrial            | P06576     | 2        | 0.000412| 1.5  |
|      | Integrin beta-3                                      | P05106     | 2        |          |      |
| 135  | Alpha- enolase                                      | P06733     | 4        | 0.0357  | −1.5 |
| 136  | Myosin-9                                            | P35579     | 5        | 0.0106  | 1.5  |
| 137  | Myosin regulatory light polypeptide 9                | P24844     | 3        | 0.0325  | 1.7  |
|      | Protein disulfide-isomerase A6                       | Q15084     | 2        |          |      |
| 138  | Endoplasmnic reticulum resident protein 29          | P30040     | 6        | 0.0104  | −1.5 |
| 139  | Peroxiredoxin-4                                     | Q13162     | 2        | 0.0362  | −1.5 |
| 140  | Tyrosine-protein phosphatase nonreceptor type 6      | P29350     | 3        | 0.0319  | −1.5 |
|      | Protein disulfide-isomerase A3                       | P30101     | 2        |          |      |
| 141  | Protein disulfide-isomerase A3                       | P30101     | 2        | 0.0125  | −1.5 |
| 142  | PDZ and LIM domain protein 1                         | O00151     | 2        | 0.00306 | 1.5  |
| 143  | ATP synthase subunit beta, mitochondrial            | P06576     | 18       |          |      |
|      | Protein disulfide-isomerase A6                       | Q15084     | 6        |          |      |
|      | 78 kDa glucose-regulated protein                    | P11021     | 4        | 0.00266 | −1.4 |
|      | Protein disulfide-isomerase                          | P07237     | 2        |          |      |
|      | Vimentin                                             | P08670     | 2        |          |      |
| 144  | Plastin-2                                           | P13796     | 15       | 0.000372| −1.6 |
| 145  | Actin, cytoplasmic 1; actin, cytoplasmic 2          | P60709; P63261 | 3    | 0.00905 | 1.5  |
| 146  | Actin, cytoplasmic 1; actin, cytoplasmic 2          | P60709; P63261 | 4    | 0.0156  | 1.8  |
| 147  | 60 kDa heat shock protein, mitochondrial            | P10809     | 9        | 0.018   | 1.5  |
| 148  | Filamin-A                                           | P21333     | 4        | 0.000123| 2.6  |
| 149  | Filamin-A                                           | P21333     | 3        | 0.0016  | 2.6  |
| 150  | Alpha-actinin-1                                     | P12814     | 7        | 0.00104 | 2.4  |
| 151  | Ficolin-1                                           | O00602     | 2        | 0.000967| −2.4 |
| 152  | Ezrin                                               | P15311     | 3        | 0.0136  | −2.4 |
| 153  | Coronin-1A                                          | P31146     | 1        | 0.00915 | −2.2 |
Table 2: Continued.

| Spot | Protein                                      | Uniprot AC | Peptides | P       | Fold |
|------|----------------------------------------------|------------|----------|--------|------|
| 154  | Alpha-actinin-1                              | P12814     | 4        | 0.00109| 2.2  |
| 155  | Fermitin family homolog 3                     | Q86UX7     | 2        | 0.000261| 2.1  |
| 156  | Filamin-A                                    | P21333     | 2        | 0.000568| 2.1  |
| 157  | Alpha-actinin-1                              | P12814     | 5        | 0.0017  | 2.0  |
| 158  | Filamin-A                                    | P21333     | 2        | 0.0123  | 1.9  |
| 159  | Ficolin-1                                     | O00602     | 2        | 0.0221  | −1.8 |
| 160  | Alpha-actinin-1                              | P12814     | 5        | 0.000516| 1.6  |
|      | Filamin-A                                    | P21333     | 2        |         |      |
| 161  | I4-3-3 protein zeta/delta                     | P63104     | 3        | 0.022   | 1.8  |
| 162  | Prohibitin                                   | P35232     | 3        | 0.0414  | −1.8 |
| 163  | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 | 2    | 0.0133  | 1.7  |
| 164  | Nucleoprotein TPR                             | P12270     | 1        | 0.00952 | 1.7  |
| 165  | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 | 5    |        |      |
|      | Filamin-A                                    | P21333     | 3        | 0.037   | 1.7  |
|      | Beta-parvin                                   | Q9HBI1     | 2        |         |      |
| 166  | Alpha-actinin-1                              | P12814     | 3        | 0.00969 | 1.7  |
| 167  | Filamin-A                                    | P21333     | 2        | 0.0109  | 1.7  |
| 168  | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 | 2    | 0.00529 | 2.8  |
| 169  | Ezrin                                        | P15311     | 2        | 0.0174  | −1.7 |
| 170  | Beta-actin-like protein 2                     | Q562R1     | 2        | 0.0226  | 1.6  |
| 171  | Filamin-A                                    | P21333     | 2        | 0.00975 | 1.6  |
| 172  | Filamin-A                                    | P21333     | 3        | 0.039   | 1.6  |
| 173  | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 | 2    | 0.0202  | 1.6  |
| 174  | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 | 3    | 0.0207  | 1.6  |
| 175  | Myosin-9                                      | P35579     | 2        | 0.0225  | 1.6  |
| 176  | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 | 4    | 0.000991| 2.2  |
| 177  | Filamin-A                                    | P21333     | 3        | 0.0131  | 1.5  |
| 178  | Filamin-A                                    | P21333     | 5        | 0.031   | 2.1  |
|      | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 | 3    |         |      |

Table 3: Functional association of the identified protein dataset with KEGG cellular pathways.

| Annotation (pathway/process)                  | XD-score | Fisher-test, q value |
|-----------------------------------------------|----------|----------------------|
| Pathogenic *Escherichia coli* infection       | 1.3719   | 3.10E − 05           |
| Proteasome                                    | 1.2793   | 4.20E − 04           |
| Glycolysis/gluconeogenesis                    | 0.9755   | 4.20E − 04           |
| Pyruvate metabolism                           | 0.9392   | 1.50E − 02           |
| Antigen processing and presentation           | 0.9051   | 5.40E − 04           |

has not yet been unraveled. There is also the possibility that a change in TCP subunits could affect the proper folding of proteins involved in hematopoiesis and its regulation and therefore may contribute to MDS pathogenesis.

Apoptosis, an important phenomenon in MDS, in a highly regulated manner removes the excess or potentially dangerous cells from the organism. The apoptotic process relies heavily on the cleavage of proteins/proteolysis. Any proteolytic pathway involved in cell death regulation must be precise; therefore, a highly regulated proteasome pathway is a good candidate for the regulation of protein composition during apoptosis [41]. Evidence of cross talk between the apoptotic pathways, HSPs, and proteasome system exists [42]; the relation of these processes is also suggested in our dendrogram analysis results (see Table 5, Group F1C). It is difficult to define a clear role or the involvement of the proteasome system in apoptosis, because in some systems proteasome activity induces apoptosis while in others it does not [42].
Table 4: Functional association of the identified protein dataset with Reactome cellular pathways.

| Annotation (pathway/process) | XD-score | Fisher-test, q value |
|-----------------------------|----------|---------------------|
| Formation of tubulin folding intermediates by CCT Tric | 3.7875 | 2.40E − 08 |
| Further platelet releaseate | 3.1660 | 5.00E − 07 |
| Prefoldin mediated transfer of substrate to CCT Tric | 2.8529 | 2.00E − 07 |
| Activation of chaperones by IRE1 alpha | 2.4375 | 1.40E − 02 |
| Postchaperonin tubulin folding pathway | 2.3845 | 9.60E − 04 |
| Chaperonin mediated protein folding | 1.6524 | 2.20E − 06 |
| Formation of ATP by chemiosmotic coupling | 1.5375 | 3.80E − 02 |
| Smooth muscle contraction | 1.4518 | 1.30E − 02 |
| Cell-extracellular matrix interactions | 1.4250 | 4.50E − 02 |
| Glycolysis | 1.3738 | 1.40E − 02 |
| p53 independent DNA damage response | 1.2026 | 9.60E − 04 |
| Stabilization of p53 | 1.1070 | 1.30E − 03 |
| Regulation of ornithine decarboxylase | 1.0779 | 1.30E − 03 |
| VIF mediated degradation of APOBEC3G | 1.0779 | 1.30E − 03 |
| Platelet degranulation | 1.0545 | 5.20E − 06 |
| SCF beta TRCP mediated degradation of EMI1 | 1.0500 | 1.40E − 03 |
| Association of Tric CCT with target proteins during biosynthesis | 1.0232 | 3.00E − 02 |

It is possible that increased proteasome activity causes the suppression of apoptosis, and this could be one of the reasons for the transformation from MDS to AML [43]. In relation to programmed cell death, researchers have identified changes in the regulatory subunits of the 19S cap complex [44, 45]. We observed changes in several proteasomal proteins and in the non-ATPase regulatory subunits of the 26S complex (Table 5, Group F1C), a part of which is the 19S cap complex [46]. We also observed changes in several HSPs, including HSP90α (identified in spot 51), whose overexpression has already been linked to apoptosis and MDS [47]. HSPs assist in proper protein folding, as molecular chaperones. They are fundamental for cell life and death decisions, and their abnormal expression is linked to oncogenesis [48]. When a protein is misfolded, HSPs are induced and associate with the misfolded protein, trying to refold it. When this process fails, the protein is ubiquitinated and determined to be processed by the proteasome. In case there are not enough HSPs or the proteasome function is impaired, proteins tend to aggregate with HSPs, ubiquitin, and proteasome to an insoluble compartment and trigger apoptosis [42, 49, 50]. HSPs can act in apoptosis at three levels: (i) in upstream mitochondria by regulating signaling molecules [51] (see Table 5, Group F1C); (ii) at the mitochondrial level by controlling mitochondrial membrane permeabilization and thus the release of cytochrome c [52] (see Table 6); and (iii) downstream of the mitochondria by affecting apoptosome formation [53] (see Table 5, Group F1C). Their role in apoptosis is also controversial; HSP function in apoptosis may be impacted by posttranslational modifications and the interaction with cochaperones (e.g., the DnaJ-family proteins identified in spot 124) [54]. The overexpression of HSPs has been shown to block apoptosis; and on the other hand, the depletion of HSPs increases sensitivity to apoptotic stimuli [55]. We observed both a decrease and an increase in normalized volumes in the spots containing HSPs (Table 2). However, from 2D SDS-PAGE data it is not possible to claim whether a change is caused by protein expression alteration or by protein post-translational modification. Therefore, there is the possibility that the HSPs identified are posttranslationally modified, differently expressed, or a combination of both. Changes in proteasomal proteins and HSPs could be involved in cells’ decisions regarding the triggering of apoptosis. Because of the inconsistency in the roles of both the proteasome and HSPs in apoptosis, we can only speculate whether the changes are the cause of the apoptosis in MDS.

In order to provide further insight into the possible PMBCs alterations in MDS patients additional to those suggested by the 2D electrophoresis data, western blot analysis was performed for the two of identified proteins: fermitin-3 and vinculin. Both the proteins were uniquely identified in the corresponding spots (without co-identified proteins) and they are both involved in particular in cell adhesion and integrin signaling [56, 57]. While vinculin was identified in five different spots with both increasing and decreasing spot normalized volumes (which strongly indicates the presence of posttranslational modifications and the alterations of individual proteoforms), fermitin-3 was observed in one spot only. The results of western blot analysis are shown in Figure 4.

It is apparent that vinculin is underexpressed in PBMCs of MDS patients when compared to the healthy donor group. Therefore, while the prevalent vinculin form is decreased in MDS there is also a minority of posttranslationally modified forms altered as estimated from 2D electrophoresis and LC-MS/MS data. Vinculin is an actin-binding protein that is involved especially in cell adhesion dynamics and cell migration [56, 58]. Downregulation of vinculin expression could
Table 5: List of the expression profile groups (A, B, and C) found in the cytosolic subproteome (F1).

| Spot | Protein                                      | Uniprot AC  |
|------|----------------------------------------------|-------------|
| 1    | Vinculin                                     | P18206      |
| 17   | Vinculin                                     | P18206      |
| 43   | Talin-1                                      | Q9Y490      |
| 48   | Vinculin                                     | P18206      |
| 59   | Tubulin alpha-1C chain                       | Q9BQE3      |
| 59   | Tubulin alpha-1A chain                       | Q71U36      |
| 59   | Tubulin alpha-4A chain                       | P68366      |
| 26   | Proteasome activator complex subunit 1       | Q06323      |
| 26   | Coagulation factor XIII A chain              | P00488      |
| 47   | Tubulin beta-1 chain                         | Q9H4B7      |
|      |                                              |             |
| 56   | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 |
| 46   | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 |
| 2    | Tubulin alpha-1C chain; tubulin alpha-1B chain | Q9BQE3; P68363 |
| 2    | Annexin A6                                   | P08133      |
| 9    | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 |
| 16   | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709; P63261 |
| 38   | Talin-1                                      | Q9Y490      |
|      |                                              |             |
| 51   | Annexin A6                                   | P08133      |
| 51   | Heat shock 70 kDa protein 1A/1B              | P08107      |
| 7    | Heat shock 70 kDa protein 1-like             | P34931      |
| 7    | Heat shock protein HSP 90-alpha              | P07900      |
| 58   | Annexin A7                                   | P09960      |
| 58   | Heat shock 70kDa protein 1-liked             | P20073      |
| 36   | Adenosylhomocysteinase                        | P23526      |
| 36   | Proliferation-associated protein 2G4         | Q9UQ80      |
| 36   | Rab GDP dissociation inhibitor beta           | P50395      |
| 36   | Alpha-enolase                                | P06733      |
| 36   | Adenylosuccinate synthetase isozyme 2        | P30520      |
| 36   | Beta-centractin                              | P42025      |
| 36   | Proliferation-associated protein 2G4          | Q9UQ80      |
| 36   | Annexin A1                                   | P04083      |
| 15   | Adenylosuccinate synthetase isozyme 2        | P06733      |
| 15   | Alpha-enolase                                | P06733      |
| 15   | Beta-centractin                              | P06733      |
| 14   | Rab GDP dissociation inhibitor beta           | P50395      |
| 14   | C-terminal-binding protein 1                 | P30520      |
| 21   | Alpha-enolase                                | P06733      |
| 3    | Alpha-enolase                                | P06733      |
| 35   | m7GpppX diphosphatase                        | Q96C86      |
| 35   | Transaldolase                                | P37837      |
| 34   | 26S proteasome non-ATPase regulatory subunit 14 | O00487    |
| 34   | Proteasome subunit alpha type-6              | P60900      |
| 23   | UPF0568 protein C14orf166                    | Q9Y224      |
| 23   | Hypoxanthine-guanine phosphoribosyltransferase | P00492    |
| 18   | Triosephosphate isomerase                    | P60174      |
Table 5: Continued.

|    |    |                        |     |
|----|----|------------------------|-----|
| 66 | Proteasome subunit beta type-3 | P49720 |
| 54 | Triosephosphate isomerase       | P60174 |
| 70 | Triosephosphate isomerase       | P60174 |
| 27 | Proteasome subunit beta type-4  | P28070 |
| 50 | Heat shock cognate 71 kDa protein | P11142 |
| 50 | Rho-related GTP-binding protein RhoC | P08134 |
| 50 | Proteasome activator complex subunit 2 | Q9UL46 |

Table 5: Continued.

![Vinculin and Fermitin-3 Proteins.png](attachment:Vinculin_and_Fermitin-3_Proteins.png)

**Figure 4:** Western blot analysis of vinculin and fermitin-3 proteins; 1, molecular weight marker (kDa); 2 and 4, pooled control samples; 3 and 5, pooled MDS patient samples.

be possibly related to the immune system dysregulation in MDS as vinculin underexpression was described in the study by Kim et al. [56] investigating proteome changes in PBMCs of patients with atopic dermatitis, a chronic inflammatory skin disease. The presence of vinculin posttranslational modifications (as indicated in this work) was also observed in the proteomic study of PBMCs collected from amyotrophic lateral sclerosis (ALS) patients; significantly higher level of vinculin nitration was observed for sporadic ALS patients compared to healthy donors [59].

Western blot analysis of fermitin-3 showed the presence of several protein forms; in addition to the uncleaved protein, various fragments of fermitin-3 were observed. The uncleaved fermitin-3 band was observed with a lower intensity in MDS patients compared to the healthy controls; this observation may suggest fermitin-3 underexpression in MDS patients. However, the total intensity of the unaltered fermitin-3 together with its fragments estimated with ImageJ software [60] showed that there is no difference between MDS patients and the healthy controls. Therefore, while fermitin-3 expression did not seem to differ between the studied groups, it was shown that there was advanced fermitin-3 fragmentation in MDS patients. Fermitin-3 is a member of the family of focal adhesion proteins exclusively expressed in hematopoietic cells [61]. Its role in adhesion is essential for the function of blood cells including leukocytes [57, 62, 63]. Thus, higher rate of fermitin-3 fragmentation observed in this work could substantially influence PMBCs function in MDS. Moreover, fermitin-3 and vinculin are known to be colocalized to hematopoietic cell adhesion structure called podosome [64]. Therefore, since vinculin and fermitin-3 are both involved in cell adhesion and integrin signaling (and thus could play an important role in clinical applications) their changes observed in this work could initiate future research efforts.

4. Conclusion

In conclusion, we have compared the peripheral blood mononuclear cell proteome of myelodysplastic syndrome patients with refractory cytopenia with multilineage dysplasia against the proteome of healthy donors using two-dimensional electrophoresis combined with mass spectrometry. Through data mining of the Reactome and KEGG databases using EnrichNet, we highlighted the possible involvement of the identified protein alterations in apoptosis, protein degradation by proteasome, heat shock protein action, and signal transduction. Western blot analysis showed substantial changes in vinculin and fermitin-3, proteins involved in cell adhesion and integrin signaling. Vinculin was found to be underexpressed in MDS patients; advanced fragmentation of fermitin-3 was shown to take place in PMBCs of MDS patients.

To the best of our knowledge, our pilot study represents the first report on the proteome changes of peripheral blood mononuclear cells in myelodysplastic syndrome.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was supported by the Czech Science Foundation P205/12/G118 and by the state project (Ministry of Health, Czech Republic) for the conceptual development of the
Table 6: List of the expression profile groups (A, B, C, D, and E) found in the membrane and membrane organelle subproteome (F2).

| Spot | Protein                                                                 | Uniprot AC |
|------|------------------------------------------------------------------------|-------------|
| 85   | Alpha-actinin-1                                                        | P12814      |
|      | 60 kDa heat shock protein, mitochondrial                               | P10809      |
| 112  | Integrin beta-3                                                        | P05106      |
|      | ATP synthase subunit beta, mitochondrial                                | P06576      |
| 130  | Synaptosomal-associated protein 23                                     | O00161      |
|      | Purine nucleoside phosphorylase                                        | P00491      |
| 107  | Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 1| P04843      |
| 142  | PDZ and LIM domain protein 1                                           | O00151      |
| 136  | Myosin-9                                                               | P35579      |

| Spot | Protein                                                                 | Uniprot AC |
|------|------------------------------------------------------------------------|-------------|
| 91   | Aconitate hydratase, mitochondrial                                      | Q99798      |
|      | Endoplasmic reticulum resident protein 29                               | P07355      |
| 94   | Heat shock protein 75 kDa, mitochondrial                                | Q12931      |
|      | Moesin                                                                  | P26038      |
| 116  | T-complex protein 1 subunit zeta                                        | P78371      |
| 132  | Calreticulin                                                            | P27797      |
| 86   | Heterogeneous nuclear ribonucleoprotein H2                              | P55795      |
| 83   | Heterogeneous nuclear ribonucleoprotein H                               | P31943      |
| 101  | 40S ribosomal protein SA                                                | P08670      |
| 124  | DnaJ homolog subfamily B member II                                     | Q9UB54      |
| 113  | Moesin                                                                  | P61978      |
| 119  | 60 kDa heat shock protein, mitochondrial                               | P10809      |

| Spot | Protein                                                                 | Uniprot AC |
|------|------------------------------------------------------------------------|-------------|
| 99   | 78 kDa glucose-regulated protein                                        | P11021      |
| 103  | 78 kDa glucose-regulated protein                                        | P11021      |
| 95   | Protein disulfide-isomerase A4                                          | P13667      |
|      | Endoplasmic reticulum resident protein 29                               | P13667      |
| 143  | ATP synthase subunit beta, mitochondrial                                | P06576      |
|      | Protein disulfide-isomerase A6                                          | Q15084      |
| 144  | Plastin-2                                                               | P13796      |
| 128  | 60 kDa heat shock protein, mitochondrial                               | P10809      |
Group F2E

| Spot | Protein                                  | Uniprot AC |
|------|------------------------------------------|------------|
| 97   | Endoplasmín                              | P14625     |
| 92   | Endoplasmín                              | P14625     |
| 90   | Transitional endoplasmic reticulum ATPase | P55072     |
| 115  | T-complex protein 1 subunit beta          | P78371     |
| 117  | Macrophage-capping protein               | P40121     |
| 102  | F-actin-capping protein subunit beta      | P47756     |

Table 7: List of the expression profile groups (A, B) found in the nuclear subproteome (F3).

| Spot | Protein                                      | Uniprot AC |
|------|----------------------------------------------|------------|
| 167  | Filamin-A                                    | P21333     |
| 156  | Filamin-A                                    | P21333     |
| 176  | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709;    |
|      |                                              | P63261     |
| 172  | Filamin-A                                    | P21333     |
| 155  | Fermitin family homolog 3                    | Q86UX7     |

Group F3A

| Spot | Protein                                      | Uniprot AC |
|------|----------------------------------------------|------------|
| 161  | 14-3-3 protein zeta/delta                    | P63104     |
| 148  | Filamin-A                                    | P21333     |
| 154  | Alpha-actinin-1                              | P12814     |
| 157  | Alpha-actinin-1                              | P12814     |
| 150  | Alpha-actinin-1                              | P12814     |

Group F3B

| Spot | Protein                                      | Uniprot AC |
|------|----------------------------------------------|------------|
| 167  | Filamin-A                                    | P21333     |
| 156  | Filamin-A                                    | P21333     |
| 176  | Actin, cytoplasmic 1; actin, cytoplasmic 2   | P60709;    |
|      |                                              | P63261     |
| 172  | Filamin-A                                    | P21333     |
| 155  | Fermitin family homolog 3                    | Q86UX7     |

Research organization (Institute of Hematology and Blood Transfusion).

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