Proteomic Case Studies of MDS in Progression: Heterogeneity and More Heterogeneity

Progresif MDS'de Proteomic Olgu Çalışmaları: Heterojenite ve Daha da Çok Heterojenite

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To the Editor,

Myelodysplastic syndrome (MDS) constitutes a diverse group of hematological disorders. Despite the heterogeneity of these disorders, there are several shared characteristics, such as apoptosis and cytopenia. These hallmarks may change over time as the disease progresses to more advanced forms or transforms into acute myeloid leukemia (AML). In spite of findings at the DNA/RNA level [1,2], our knowledge at the protein level is limited. Given the high heterogeneity of MDS and other factors (e.g., comorbidities, treatment regimen) that influence changes at the protein level, uniform cohorts of patients (e.g., specific MDS subgroups, specific responses) are usually selected to eliminate the influence of these factors on results. The question is how many of the changes observed in such studies would be relevant for all individuals if the specific cohort criteria were removed. The aim of this work was to discover how the protein changes observed in population-based proteomic studies manifest in individual patients during MDS progression or transformation.

Patients with MDS during transformation to AML (n=6) or progression to refractory anemia with excess blasts type 2 (RAEB-2; n=3) were selected with an average of 5.3 samples per patient. Plasma proteomes were analyzed by 2D electrophoresis, proteins were identified by mass spectrometry, and protein levels were measured by ELISA as previously described in detail [3]. All samples were obtained with the written consent of the patients and were analyzed in accordance with the Ethics Committee regulations of the Institute of Hematology and Blood Transfusion in Prague.

Fifty spots that changed during transformation or progression were found (spot fold value of ≥ 1.2, $p<0.05$) and their proteins were identified. The identification was consistent with the results of previous proteomic studies [3,4,5,6], and comparisons of the identified proteins with the results of previous studies are summarized in Table 1. Surprisingly, only a few proteins changed in most individual cases. The majority of proteins were altered in small subgroups of patients and, moreover, these subgroups did not overlap. This is in contrast to population-based proteomic studies of various MDS subgroups, among which most of the identified proteins were similar and the main source of the observed differences was fold change [3,4,5,6].

Spots of only three proteins were found to be altered in the majority of cases: retinol-binding protein 4 (RBP4; decreased in all cases), alpha-2-HS-glycoprotein (AHSG; decreased in 8 of 9 cases), and leucine-rich alpha-2-glycoprotein (LRG; increased in 7 of 9 cases). These proteins were identified as potential markers in previous population-based studies. In subsequent research [7,8], modifications of RBP4 and AHSG were proposed as the main sources of their alterations. We used ELISA to estimate plasma levels of LRG, afamin, and pigment epithelium-derived factor, which changed during transformation and progression as observed by 2D electrophoresis. No correlation of their plasma levels with transformation or progression was observed; this is another observation supporting the crucial role of modifications.

One of the main problems in MDS proteomics remains MDS heterogeneity, which limits the sizes of the studied cohorts. Although this is also a limitation of the present study, we showed that the issue of heterogeneity is even more complex at the protein level than it appears to be from the results of population-based studies. Considering the results of this study and population-based studies published to date, the most promising MDS marker candidates (RBP4, AHSG, and LRG) remain the same. However, the discrepancy between the results obtained from these two types of approaches may lead to a refinement in focus in the search for clinically valuable protein markers. The alterations selected in population-based studies may be very useful for MDS pathophysiology research, but their roles as clinical markers are limited because their changes are reflected in only small cohorts of patients during MDS transformation and progression.

Table 1. List of identified proteins.

			Identified in the study: + yes/-no			
Identification	Accession number: UniProt	Peptides	RCMD [3]	RAEB-1 [4]	RA-RARS [6]	RAEB-2 [5]
Afamin	P43652	18	+	-	+	-
Alpha-1-antichymotrypsin	P01011	16	+	+	+	+
Alpha-1B-glycoprotein	P04217	7	+	+	+	+
Alpha-2-HS-glycoprotein	P02765	6	+	+	+	+
Angiotensinogen	P01019	11	+	+	+	+
Antithrombin-III	P01008	6	+	+	+	-
Apolipoprotein A-I	P02647	4	+	+	+	+
Apolipoprotein A-IV	P06727	26	+	-	+	+
Apolipoprotein E	P02649	16	+	-	+	-
Beta-2-glycoprotein 1	P02749	11	+	+	+	+
Ceruloplasmin	P00450	8	+	+	-	-
Clusterin	P10909	4	+	+	+	+
Complement C2	P06681	10	+	-	-	-
Complement C4-A; Complement C4-B	P0C0L4; P0C0L5	22	+	+	+	-
Complement C5	P01031	4	+	+	+	-
Complement factor B	P00751	26	+	+	-	-
Complement factor H	P08603	41	-	-	-	-
Complement factor I	P05156	9	+	+	+	+
Corticosteroid-binding globulin	P08185	6	+	+	+	+
C-reactive protein	P02741	5	-	+	+	+
Ferritin light chain	P02792	4	+	-	-	-
Fibrinogen beta chain	P02675	20	-	-	-	-
Fibrinogen gamma chain	P02679	19	-	+	-	-
Haptoglobin	P02749	2	+	+	-	+
Hemopexin	P02790	13	+	+	+	+
Histidine-rich glycoprotein	P04196	4	+	+	+	+
Insulin-like growth factor-binding protein complex acid labile subunit	P35858	14	-	-	+	-
Inter-alpha-trypsin inhibitor heavy chain H2	P19823	15	+	+	-	-
Kallistatin	P29622	4	-	+	+	-
Kininogen-1	P01042	9	+	+	+	+
Leucine-rich alpha-2-glycoprotein	P02750	12	+	+	-	+
Pigment epithelium-derived factor	P36955	14	+	+	-	-
Protein AMBP	P02760	6	-	+	+	+
Prothrombin	P00734	13	+	+	+	+
Retinol-binding protein 4	P02753	10	+	+	+	-
Serum albumin	P02768	9	+	+	+	+
Serum amyloid P-component	P02743	5	-	+	+	-
Tetranectin	P05452	10	-	+	+	+
Vitamin D-binding protein	P02774	6	+	+	+	-
Zinc-alpha-2-glycoprotein	P25311	15	+	+	-	+

RCMD: Refractory cytopenia with multilineage dysplasia; RAEB-1: refractory anemia with excess blasts type 1; RAEB-2: refractory anemia with excess blasts type 2; RA-RARS: refractory anemia and refractory anemia with ringed sideroblasts.

Keywords: Myelodysplastic syndrome, Plasma proteome, Proteomics

Anahtar Sözcükler: Myelodisplastik sendrom, Plazma proteomu, Proteomikler

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Ethics

Informed Consent: Obtained.

Authorship Contributions

Concept: K.P., P.M.; Design: K.P., P.M.; Data Analysis or Interpretation: K.P., P.M., K.P., J.C., P.M.; Writing: K.P., J.C., P.M.

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