ABSTRACT

Myelodysplastic syndromes (MDS) are a heterogeneous group of bone marrow (BM) disorders characterized by ineffective haematopoiesis, BM dysplasia, and peripheral blood cytopenia. In recent years, substantial progress has been made towards understanding the molecular pathogenesis of MDS that has brought new possibilities in MDS diagnostics, prognostics, and treatment.

Small noncoding RNAs (sncRNAs), especially microRNAs (miRNAs), are in the field of scientific interest in terms of their expression, function, role in disease development, and potential utilization as disease biomarkers. Special attention has been focused on extracellular sncRNAs present in blood circulation, so called 'circulating' sncRNAs, which may become easily accessible biomarkers of disease state or risk of progression.

We have conducted several studies on intracellular and extracellular sncRNA profiles of CD34+ BM cells and blood plasma, respectively, from MDS patients using microarrays or next generation sequencing (NGS). We aimed to identify specific sncRNA profiles associated with MDS and search for sncRNA biomarkers predictive of the patient prognosis and response to treatment with azacitidine (AZA). Another goal was to characterize and compare circulating sncRNA profiles of two different extracellular materials, total plasma and plasma-derived extracellular vesicles (EVs), in order to determine their usefulness as sources of MDS biomarkers.

Initially, using microarrays, we identified significantly lower levels of miR-27a-3p, miR-199a-5p, and miR-223-3p in total plasma of higher-risk MDS patients compared to lower-risk MDS patients. Further analyses indicated that the low levels of miR-223-3p and miR-451 are associated with unfavourable overall survival (OS) and progression-free survival, respectively.

Using NGS, we found other deregulated sncRNAs, including non-miRNA species, between early and advanced stages of MDS. We observed increased levels of many circulating miRNAs related to haematopoiesis (e. g. miR-103a-3p, miR-103b, miR-107, miR-221-3p, miR-221-5p, and miR-130b-5p) and miRNAs located in chromosomal region 14q32 (e.g. miR-127-3p, miR-154-5p, miR-323b-3p, miR-382-3p, miR-409-5p, and miR-485-3p) in early MDS compared to advanced MDS.

We defined a signature of four sncRNAs (miR-1237-3p, U33, hsa_piR_019420, and miR-548av-5p) whose EV levels were the most significantly associated with OS. Further, a combined score of five plasma miRNAs (miR-423-5p, miR-126-3p, miR-151a-3p, miR-125a-5p, and miR-199a-3p) was determined as a predictor of response to AZA treatment. In CD34+ BM cells, the high level of miR-17-3p and low levels of miR-100-5p and miR-133b before treatment were associated

with favourable overall response rate to AZA therapy. Moreover, miR-100-5p was found as a predictor of survival, its low level before treatment associated with favourable OS in AZA treated patients.

Regarding the two extracellular materials, total plasma and EVs, hierarchical cluster analysis showed that RNA content of EV samples is more homogeneous than that of total plasma samples. Further, substantially higher number of deregulated sncRNAs between these two materials was found in MDS patients than in control counterparts.

In conclusion, our results demonstrate distinct sncRNA profiles in total plasma, EVs, and CD34+ cells of MDS patients. These profiles are specific for distinct MDS stages and may predict patient outcome. We identified several sncRNAs, mostly miRNAs, that are associated with patient survival and response to AZA therapy and thus, may be considered as potential biomarkers of the disease.