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636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Transposable Elements Are Differentially Expressed in MDS Stem Cells in a Disease-Stage-Specific Manner

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Introduction

Transposable elements (TEs) are widely spread across the human genome. Although TEs have been implicated in the pathogenesis of hematological malignancies, their expression in myelodysplastic syndromes (MDS) has not yet received extensive analysis. In the present study, we performed RNA sequence analysis of bone marrow stem and progenitor cells from patients with MDS and secondary acute myeloid leukemia (AML) and profiled differentially expressed TEs.

Methods

Bone marrow samples were obtained from each patient at the time of diagnosis. Normal bone marrow (NBM) samples from four healthy individuals were purchased from Lonza and processed in the same manner. After separation of $CD34^+$ cells using magnetic beads, we purified lineage marker-negative (Lin^-) $CD34^+$ $CD38^-$ stem cells and Lin^- $CD34^+$ $CD38^+$ progenitor cells by cell sorting. The sorted cells were subjected to RNA-seq analysis using oligo-dT primers, which can capture poly-adenylated TE transcripts. Each read was aligned to the reference genome, then the annotation of the transcripts was performed using RepeatMasker, a program that screens DNA sequences for interspersed repeats and low-complexity DNA sequences (https://www.repeatmasker.org). Read counts for individual TEs were aggregated to the level of TE class, family, and subfamily according to hierarchies defined by the human RepeatMasker (repClass, repFamily, and repName, respectively). To generate raw counts of individual TE regions in RepeatMasker, the bedtools map function (version 2.27.1) was used with bed files of each sample. Normalization and significant expression differences were detected using DESeq2 (version 2.2.1). Differentially expressed TEs were defined using the cutoff of adjusted P values ($q < 0.05$). DESeq2 normalized counts were

obtained from read counts by considering sequence depth, gene length, and RNA composition. Normalized counts were z-score scaled, and then subjected to uniform manifold approximation and projection (UMAP).

Results

Thirty-two patients were included in the present study. According to the 2016 revision to the World Health Organization classification (Arber DA, et al. Blood. 2016; 127: 2391-2405), 13 patients (40.6%) were diagnosed with MDS with multilineage dysplasia (MDS-MLD), 15 patients (46.9%) with MDS with excess blasts (MDS-EB), and 4 patients (12.5%) with AML with myelodysplasia-related changes (AML-MRC). We found that 26 and 17 repName TEs were significantly up-regulated in stem and progenitor cells compared with their NBM counterparts, and the majority of them belonged to LTRs, followed by DNA transposons. Since each LTR has multiple individual regions where similar sequences are scattered, LTR expression was further distinguished by the genomic locations of individual LTRs.

Comparison of MDS-MLD, MDS-EB, and AML/MRC stem and progenitor cells to NBM counterparts revealed that more LTRs were differentially expressed in stem cells than in progenitor cells. Among them, many of the up-regulated LTRs were shared among different disease types (MDS-MLD, MDS-EB, and AML-MRC) in both stem and progenitor cells. AML-MRC had the largest number of differentially up- and down-regulated LTRs in stem cells. MDS-EB had the largest number of differentially up-regulated LTRs in progenitor cells, while only a small portion of LTRs were down-regulated in progenitor cells. LTRs at individual regions were differentially expressed in a more disease-specific manner, particularly in stem cells. Accordingly, UMAP plots that visualized the expression profiles of LTRs at individual regions showed that AML-MRC stem cells form a unique cluster, discriminating AML-MRC from MDS.

To further understand the transcriptional reprogramming of LTRs during the disease progression from MDS to secondary AML, we analyzed the expression of differentially expressed LTRs at individual regions specific to AML-MRC by K-means clustering. They were subdivided into four clusters, of which Clusters 1, 3, and 4 showed gradual upregulation during disease progression, while Cluster 2 showed comparable upregulation at MDS and AML-MRC stages.

Conclusions

Our results suggest that specific LTRs behave in a disease-status and cell-type-specific manner in MDS and during leukemic transformation, providing fundamental information for understanding the pathogenesis of MDS.

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