

P446 COMPREHENSIVE CHARACTERIZATION OF DIFFERENTIAL GENE EXPRESSION AND ISOFORM USAGE IN SPLICING FACTOR-MUTATED ACUTE MYELOID LEUKEMIA USING LONG-READ SEQUENCING

Topic: 3. Acute myeloid leukemia - Biology & Translational Research

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Background:

Splicing factor (SF) mutations are recurrent events in Acute Myeloid Leukemia (AML) and identified in up to 20 % of adult patients. SF mutations lead to differential gene expression and alternative splicing events that contribute to pathogenesis. The characterization of these differential gene expression, splicing events and alternative isoforms using current standard sequencing approaches is difficult and requires bioinformatic reconstruction of the original molecules due to the short read length.

Aims:

To gain further insights into the functional consequences of SF mutations we applied long-read sequencing in a large cohort of AML patients with and without SF mutations to achieve an unprecedented level of accuracy.

Methods:

We analyzed 93 intensively treated adult AML patients enrolled in the multicenter German AML Cooperative Group (AMLCG) 2008 trial or Registry (DRKS00020816) between 2008 and 2019 and 6 healthy controls. Patients and controls were profiled for molecular features via targeted sequencing platforms. The ONT PromethION platform was used for transcriptome sequencing from full-length poly-A+ RNA. Samples were sequenced to a depth of at least 5 million reads and had to pass several quality parameters. 65 AML samples with the most frequent SF mutations passed our QC criteria including *SRSF2* p.P95H (n=19), *SRSF2* p.P95L (n=10), *SRSF2* p.P95R (n=5), *SRSF2* other (n=1), *SF3B1* (n=12), *SF3A1* (n=2), *U2AF1* (n=7) and *ZRSR2* (n=9). As control 28 AML samples without SF mutation and 6 samples from donors without myeloid malignancy or clonal hematopoiesis were analyzed. For the identification of differential gene expression, alternative transcripts, and novel isoforms we used the state-of-the-art workflow wf-transcriptomes epi2me labs from Oxford Nanopore. This pipeline provides a differential expression part based on DESeq and an isoform analysis part based on DEXSeq.

Results:

The initial analysis focused on the homogenous subgroup of male AML patients with the most common SF mutation *SRSF2* p.P95H (n=19) and compared those to 16 male AML patients without SF mutations. Our analysis revealed

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220 upregulated and 185 downregulated genes with at least two fold change and a FDR of ≤ 0.05 (Figure 1A).

The DEXSeq analysis revealed also remarkable changes on splicing and alternative isoform expression between patients with hot spot mutation *SRSF2* p.P95H and AML patients without SF mutation. We found 13,836 transcripts differentially expressed corresponding to 4,371 genes. A p-value < 0.05 was defined as differentially expressed. Gene Ontology showed enrichment of gene sets associated with RNA-processing, histone modification and several other cellular processes.

After the stageR analysis, 1,727 differentially used transcripts remained, which can be assigned to 66 different gene loci. Figure 1B shows an example of a sashimi plot for one of these genes.

Summary/Outlook:

To our knowledge, this is the first large scale analysis of splicing and isoform patterns in AML by long read sequencing. Our study demonstrates that individual splicing mutations result in unique patterns of differential gene expression, splicing and isoform profiles that are remarkably consistent between patients. Given the stability of the profiles and the early appearance of SF mutations as founding clones these findings may help to identify vulnerabilities and targets not only for the treatment of AML but also applying for premalignant myeloid states like CHIP, CCUS and other myeloid neoplasia like MDS.

Comparative analyses of AML samples with other SF hot-spot mutations in *SRSF2*, *SF3A1*, *SF3B1*, *U2AF1*, *ZRSR2* are currently ongoing as well as comparisons to healthy control samples.

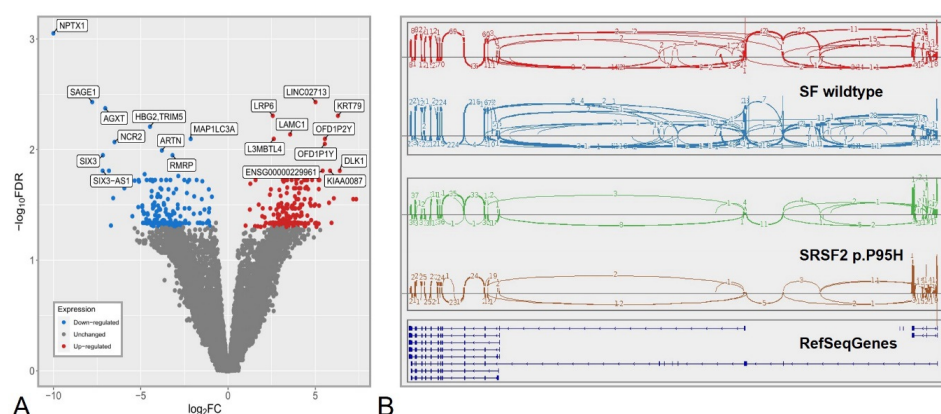


Figure 1 (A) Volcano plot of differentially expressed genes comparing *SRSF2* p.P95H with non-SF mutated AML samples. Labels indicate the top 10 up- (red) and down-regulated (blue) genes.

(B) Sashimi plot of 4 exemplary AML samples of the gene *ASNS*. Samples in red and blue are non-SF mutated, green and brown are *SRSF2* p.P95H mutated. The DEXSeq analysis shows a clear difference in isoform usage between the SF mutant and SF-wildtype AML samples.

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