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602.MYELOID ONCOGENESIS: BASIC

Differential Isoforms Signature of Splicing Factor Mutant Myeloid Neoplasia

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Exclusivity of splicing factor (SF) mutations is a near-dogma in myeloid neoplasia (MN). Such exclusivity is often reflected in morphopathologic features as in the case of *SF3B1* mutations associated with ringed sideroblasts. Consequences of SF mutations are hard to reconcile given the central role of RNA splicing in normal physiology and the multitude of alternative splicing changes occurring upon mutations. Historically, distinct gene isoforms play key roles in hematopoiesis and might be tumor associated.

We performed global transcript level expression analysis to identify SF specific differentially expressed isoforms associated with isoform disequilibrium. Such analysis prompted to the distinction between common and exclusive isoforms possibly discerning novel mechanistic insights in the pathogenesis of SF mutant MN. Of particular importance is the discovery of novel isoforms not expressed in normal condition.

Total RNA isolated from 100 whole bone marrows of MN patients with six SF mutations (*DDX41*, *PRPF8*, *SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*) and healthy subjects was subjected to RNASeq library prep (Kapa RNAHyperPrep Kit with rRNA depletion). Transcript abundance was generated using spliced alignment to the genome by *STAR* followed by *Salmon*-basedquantification. We then applied bootstrapping to capture variation in abundance estimates since transcript-level quantification is amenable to variation, in contrast with gene level, due to the difficulty of unique mapping of short read sequences. Differential isoform testing was done using *edgeR* accounting for overdispersion due to isoform uncertainty. Mutant samples were selected with VAF >15% for better estimation of isoforms *vs* clonality.

Differential SF isoform usage was defined in comparison to healthy controls. In total, 1681 unique transcripts in 1418 unique genes (positively/negatively dysregulated) at a threshold of .05 *P*-value and absolute logfold change of 2.0 were selected. Among patients with SF mutations, patients carrying sole *U2AF1* had the highest number of distinct isoforms (n=189), which accounted for about 20% of isoforms in the entire cohort of *U2AF1* mutant patients. *DDX41* mutations had the second highest (n=161), while *ZRSR2* had the lowest number of distinct isoforms(n=48). We categorized the features in groups I1-I6 according to the number of SF sharing isoforms. I1 indicates the group of patients carrying isoforms which where distinct per each SF (**Fig1**). *U2AF1* ^{I1} included genes previously reported to be associated with *U2AF1* (*e.g.*, *GNAS*) and genes restricted to neutrophil degranulation. *DDX41* ^{I1} was enriched in innate immune response gene isoforms including HLA-DQA1 and *SAMHD1* and known genes predisposing to myeloid and blood disorders (*e.g. CSF1R*, *RPL15*). *SRSF2* isoform signature was mainly characterized by upregulation of INF-gamma and beta, IFN-induced OAS1 and TLR response pathways (*e.g.*, IRAK2). Specific isoforms were shared between SFs. *SF3B1* shared 35 isoforms with *PRPF8* and 8 with *SRSF2*, including Stathmin1, a gene expressed in high proliferative MN phenotypes. Different isoforms in the same gene were also associated with different groups. For instance, DDX5 isoform-219 producing a 65-residues transcript was shared between *PRPF8* and *SF3B1* (I2) while isoform-234 producing 614-residues transcript was shared between *PRPF8*, *DDX41*, and *ZRSR2* mutants (I3). Beside the unique isoform signature per each SF, we imputed any differential isoform changes in myeloid genes often associated

with SF mutations. Isoforms for the TET triad were analyzed and resulted in a 110 residues transcript (TET2-208) significantly downregulated (FDR < .05) in all SF, except *SRSF2*.
We then focused on the discovery of exclusive isoforms. In total, 115 unique transcripts (lncRNAs, 20; protein coding, 95) were expressed in all SF mutant patients but not expressed in healthy controls. GDF15 isoform-201 encoding a 308-residues transcript was detected in *SF3B1*, *DDX41*, and *ZRSR2* mutants. LncRNAs have gained attention in regulating gene expression in MN. We found 9 novel lncRNA transcripts in *DDX41*, *SF3B1*, *PRPF8*, *U2AF1* and *ZRSR2* mutants, with one being common in all of them (ENSG00000273445 chromosome 2p11.2).
In sum, our study focusing solely on transcript level analysis describes the first isoform landscape of SF mutant MN and launches the idea that isoform analysis might shed light on the diversity and uniqueness of SF mutations.

Disclosures Maciejewski: *Regeneron:* Consultancy, Honoraria; *Alexion:* Membership on an entity's Board of Directors or advisory committees; *Novartis:* Honoraria, Speakers Bureau; *Omeros:* Consultancy.

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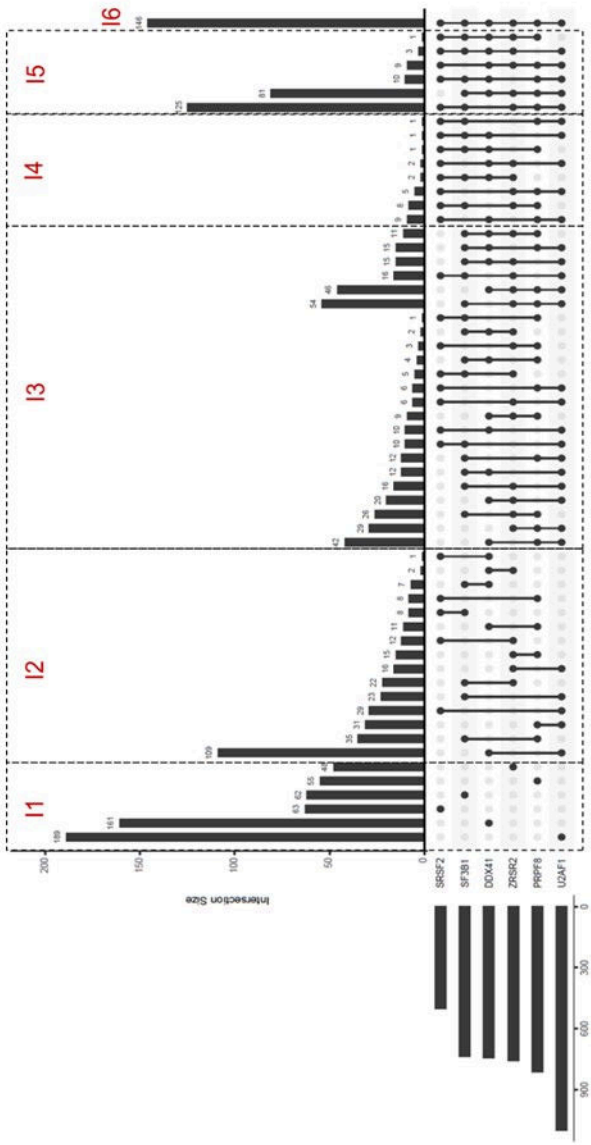

Figure 1. UpSet plot visualizing intersecting sets of isoforms across splicing factor mutations. Six groups were identified (I1-I6) indicating distinct isoforms per each splicing factor (I1) and isoforms shared in two (I2), three (I3), four (I4), five (I5) and six (I6) splicing factors. Numbers on the top of each bar indicate the number of isoforms (distinct or shared). Black dots indicate the match of the isoforms with each splicing factor. Bar graph (bottom left) represents the size (number) of all isoforms per each splicing factor in all groups.

Figure 1