AWARD NUMBER: W81XWH-17-1-0093

TITLE: Mutations in the Spliceosomal Gene ZRSR2 in Myelodysplastic Syndromes

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REPORT DATE: July 2020

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2020	Final	1Apr2017-31Mar2020

4. TITLE AND SUBTITLE

Mutations in the Spliceosomal Gene ZRSR2 in Myelodysplastic Syndromes

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0093

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Myelodysplastic syndromes (MDS) are characterized by recurrent somatic alterations often affecting components of RNA splicing machinery. Mutations of splice factors SF3B1, SRSF2, ZRSR2 and U2AF1 occur in >50% of MDS. To assess the impact of spliceosome mutations on splicing and to identify common pathways/genes affected by distinct mutations, we performed RNA-sequencing of 32 MDS bone marrow samples harboring solely spliceosome mutations (including SF3B1, SRSF2 P95 or small deletions, U2AF1 S34 or Q157 hotspot mutations and ZRSR2 truncating alterations), but devoid of other co-occurring mutations. We uncover the landscape of splicing alterations in each splice factor mutant MDS and demonstrate that SRSF2 deletions cause highest number of splicing alterations compared with other spliceosome mutations. Although the mis-spliced events observed in different splice factor mutations were largely non-overlapping, a subset of genes, including *EZH2*, were aberrantly spliced in multiple mutant groups. Pathway analysis revealed dysregulated biological processes including RNA splicing and transport as well as several signaling cascades, which were observed repeatedly in different mutant groups, suggesting converging biological consequences downstream of distinct spliceosome mutations.

15. SUBJECT TERMS

ZRSR2, myelodysplastic syndromes (MDS), splicing factor mutations, U12-type intron splicing

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	12	19b. TELEPHONE NUMBER *(include area code)*

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Technical Report DoD Award W81XWH-17-1-0093,BM160010

1. INTRODUCTION

Discovery of frequent mutations in splicing factor genes (especially *SF3B1*, *U2AF1*, *SRSF2* and *ZRSR2*) in myelodysplastic syndromes (MDS) chronic lymphocytic leukemia (CLL), and to lesser extent in other blood cancers including acute myeloid leukemia (AML), uncovered an unexpected role for RNA splicing in hematopoietic transformation. Although significant advances have been made in understanding the role of *SF3B1*, *U2AF1*, *SRSF2* mutations in MDS pathogenesis, very little is known about the contribution of *ZRSR2* mutations. Several attributes differentiate the X-linked gene *ZRSR2* from the other splicing factors: 1) Mutations in *ZRSR2* occur across the full length of the transcript and are often stop or frameshift mutations, a pattern consistent with loss-of-function; mutations in other splicing factors occur at highly restricted residues, suggesting an alteration of function. 2) While complete loss of either *SF3B1*, *U2AF1* or *SRSF2* is detrimental to cells, loss of *ZRSR2* is not lethal but rather affects essential developmental processes. 3) ZRSR2 primarily targets genes with U12-type introns, whereas the other splicing factors regulate splicing of U2-type introns. Despite these differences, *ZRSR2* mutations are largely mutually exclusive with mutations of *SF3B1*, *SRSF2* and *U2AF1*, and like the other splicing factor mutations, MDS cells with mutated *ZRSR2* might be preferentially sensitive to inhibition of splicing. Although rare, U12-type introns are found in genes involved in vital cellular processes, and mutations in core U12-type spliceosomal genes are associated with several diseases including MDS and AML. We showed that knockdown of *ZRSR2* in leukemic cell lines (TF1, K562) led to slower growth rates, reduced colony formation, and decreased tumor size in NSG mice. Silencing (shRNA) of *ZRSR2* in normal human hematopoietic $CD34^{+}$ progenitors altered the differentiation of erythroid and myeloid cells, the major cell lineages affected in MDS. We preformed RNA-seq on MDS samples harboring *ZRSR2* mutations and isogenic TF1 and K562 cell lines with *ZRSR2*-knockdown (shRNA). Strikingly, in all *ZRSR2* mutant samples and ZRSR2 silenced cells, aberrant splicing primarily affected U12-type introns, including those found in genes implicated in myeloid malignancies, such as E2F transcription factors, MAP kinases, *PTEN*, and *BRAF*. Our research identified a specific role for ZRSR2 in RNA splicing and highlighted dysregulated splicing of U12-type introns as a hallmark of ZRSR2 mutations in MDS.

2. KEYWORDS

ZRSR2, myelodysplastic syndromes (MDS), splicing factor mutations, U12-type intron splicing

3. ACCOMPLISHMENTS

Gene-specific and lineage-specific splicing alterations in *ZRSR2*-deficient hematopoietic cells.

To investigate the role of ZRSR2 in normal hematopoiesis and leukemogenesis, we generated a *Zrsr2* KO ($Zrsr2^{-/Y}$) murine model. Male $Zrsr2^{-/Y}$ mice were obtained at expected frequency and did not display apparent morphological abnormalities. RNA-seq analysis of total marrow revealed moderate increased mis-splicing of U12-type introns in KO compared with WT cells. Detail phenotypic analysis of hematopoietic compartments of both young (8-weeks old) and old (1-year old) $Zrsr2^{-/Y}$ mice showed no significant difference compared to $Zrsr^{+/Y}$ mice, suggesting that the increased mis-splicing is insufficient to drive dysplasia. For example, no difference in peripheral blood cell counts, proportion and absolute numbers of hematopoietic stem cells (HSCs) including long-term and short-term HSCs cells, or the numbers of myeloid precursors was noted (Fig. 1). Similarly, frequency of mature myeloid cells in bone marrow and spleen of $Zrsr2^{-/Y}$ mice were largely conserved (data not shown). Competitive bone marrow transplantation assays with $Zrsr2^{-/Y}$ hematopoietic progenitor cells showed that their multi-lineage reconstitution potential is comparable with that of wild-type (WT) ($Zrsr2^{+/Y}$) cells (Fig. 2). Despite the lack of an obvious disease phenotype, our preliminary studies indicate that analysis of mis-splicing events in bone marrow of $Zrsr2^{-/Y}$ mice can provide valuable insights into the importance of ZRSR2-induced splicing in hematopoietic differentiation.

To investigate the consequences of *Zrsr2* deficiency on splicing in specific hematopoietic lineages, we performed RNA-seq on hematopoietic populations from bone marrow of $Zrsr2^{-/Y}$ mice. In preliminary analysis on FACS-sorted common myeloid progenitors (CMPs), granulocyte monocyte progenitor (GMPs), and megakaryocyte

erythroid progenitor (MEPs), we observed increased retention of U12-type introns in *Zrsr2*$^{-/Y}$ cells compared with *Zrsr2*$^{+/Y}$ cells (Fig. 3).

While loss of *ZRSR2* in human cells alters hematopoietic differentiation and is associated with myeloid malignances, *Zrsr2*$^{-/Y}$ mice appear to have normal hematopoiesis. *ZRSR2* has a closely related paralog, *ZRSR1*; human *ZRSR1* is a pseudogene of *ZRSR2*; murine *Zrsr1* is a protein coding gene that likely compensates for loss of *Zrsr2*. To generate murine bone marrow cells that more closely mimic human expression of *ZRSR* genes, we infected young marrow progenitors (Lin$^-$Kit$^+$) from *Zrsr2*$^{-/Y}$ and *Zrsr2*$^{+/Y}$ mice with either shRNA-*Zrsr1*-RFP (sh*Zrsr1*, optimized in NIH3T3 cells) or control shRNA-GFP (shGFP). RFP/GFP-tracking viability assays showed that knockdown of *Zrsr1* in WT (*Zrsr2*$^{+/Y}$) cells was associated with reduction in cell survival, which is enhanced when *Zrsr1* was knockdown in *Zrsr2* KO (*Zrsr2*$^{-/Y}$) cells. Knockdown of *Zrsr1* in WT cells also decreased formation of colonies in methylcellulose colony assays, with a further decrease occurring in *Zrsr2* KO cells with *Zrsr1*-knockdown cells. These results suggest that *Zrsr1* plays an important role in murine hematopoiesis, and that silencing of both *Zrsr1* and *Zrsr2* might be required to mimic the loss-of-function mutations of *ZRSR2* found in human patients. We performed RNA-seq analysis on RFP and GFP FACS-sorted cells and compare the results to our human RNA-seq data. Results showed that the U12-type intron mis-spicing in bone marrow cells from *Zrsr2*$^{-/Y}$ mice is enhanced by knockdown of *Zrsr1* (Fig. 4). We verified increased expression of selected transcripts containing retained introns by qPCR.

To learn more about the mechanisms associated with ZRSR2-induced U12-type splicing, we looked for ZRSR2 binding partners that can affect its splicing activity. To identify ZRSR2 interacting proteins, we fused the N-terminus and C-terminus of ZRSR2 to both biotin ligase (BirA*) and FLAG, generating two ZRSR2 fusion vectors. We transfected the fusion proteins and control empty vectors into *Flp*-In™ *293 T-REx* cells and established stable and inducible isogenic cell lines ± ZRSR2-BirA*-FLAG. C-terminus and N-terminus tagged-ZRSR2 proteins were induced by either doxycycline or doxycycline/biotin and immunoprecipitated with either FLAG or biotin, respectively. ZRSR2 interacting partners were identified by mass spectrometry. In a polit experiment, known ZRSR2 partners including members of the SF3B complex, U2AF1, U2AF2 and SRPK1 were identified, and their interaction with ZRSR2 was verified by immunoprecipitation and western blotting. Novel candidate interacting partners also included multiple proteins involved in RNA splicing/processing (e.g. PNN, SON, DDX42). Among the interesting novel ZRSR2 interactors are C1QBP, which is involved in metabolism and tumorigenesis and is known to associate with the splicing factor SRSF1 and inhibit its binding to RNA, and CCDC97 whose function is largely unknown. An interaction between ZRSR2 and C1QBP and CCDC97 was confirmed (Fig. 5). CCDC97 is predicted to bind SF3B family members (STRING database), and genome wide association studies (GWAS) suggest it is linked to hematopoietic cell function and tumorigenesis. Our studies showed that knockdown of CCDC97 in TF1 cells impaired cell growth and colony-formation, as well as global splicing (Fig. 6).

Investigate shared cancer promoting mechanisms for *ZRSR2* and other splicing factor mutations.

RNA-seq data by us and others showed that multiple splicing factor mutations, including *ZRSR2* widely target genes involved in DNA damage response, making the DNA damage response a top candidate for a convergent mechanism in MDS with spliceosome mutations. To examine whether *ZRSR2*-deficient cells exhibit increased levels of DNA damage, we measured DNA damage with a pan-DNA damage marker H2AX-*pSer*139 (γH2AX) antibody, in leukemic cell lines (TF1, K562) stably transfected with either ZRSR2-shRNA or Cont-shRNA. Immunostaining showed a small increase in γH2AX signals in *ZRSR2*-knockdown cells compared to control cells.

Elevated levels of DNA damage in cells with certain splicing factor mutations is mediated at least in part through induction of R-loops in a splicing independent manner. To determine whether increased R-loop formation occurs in *ZRSR2*-knockdown cells, we measured R-loops with S9.6, an antibody that specifically recognizes RNA/DNA hybrids. Genomic DNA from stably transfected ZRSR2-shRNA or Cont-shRNA cells was immunoprecipitated with monoclonal S9.6 antibody, spotted on nitrocellulose membranes and stained with a secondary antibody. Results showed higher levels of R-loops in HEK293T, K562 and TF1 *ZRSR2*-knockdown cells (Fig. 7). To strengthen these results, we used HEK293T cells stably transfected with either wild-type (WT) or mutant (D210N or WKKD) RNASEH1, an enzyme that specifically degrades the RNA in R-loops. D210N

mutation abolishes catalytic activity of RNASEH1; WKKD mutation abolishes both binding and catalytic activities of RNASEH1 and is used for negative control. We generated HEK293T cells stably expressing either V5-tagged RNASEH1 WT, D210N, or WKKD (vectors were a gift from Dr. D Zhang) and further transduced the cells with either ZRSR2-shRNA or Cont-shRNA. Dot blot analysis with S9.6 showed elevated R-loops in ZRSR2-depleted RNASEH1 WT cells, which further increased in D210N expression cells, and not in WKKD expression cells.

ATR-CHK1 and ATM-CHK2 are two crucial pathways controlling the DNA damage response pathway. Induction of R-loops by mutated splicing factor activates the ATR but not ATM pathway. Cells with *U2AF1* mutation show increased sensitivity to ATR inhibition, which is depended on the presence of R loops. Similarly, we found that ATR inhibitor VE-821 reduced the viability of *ZRSR2*-knockdown TF1 cells more than that of control cells.

Activation of innate immune signaling was proposed as another unifying mechanism mediating the effects of splicing factor mutations in MDS. To test whether loss of ZRSR2 renders cells more sensitive to inflammatory stimuli and NF-κB activation, we treated TF1 *ZRSR2* WT and knockdown (shRNA) cells with either lipopolysaccharide (LPS) or tumor necrosis factor α (TNFα) and measured levels of p65 phosphorylation (p-p65), a marker for NF-kB activation. Western blot analysis showed increased p-p65 levels in *ZRSR2* knockdown cells compared with control cells following TNFα but not LPS treatment. The reason for specific induction by TNFα and not LPS is unclear. A possible explanation is that loss of ZRSR2 induces changes in genes that respond to TNFα but not to LPS stimulation.

Though several studies identified common mechanisms in MDS patients with different splicing factor mutations, these studies either understudied or excluded *ZRSR2*. Also, many of the patient samples contained additional mutations in known myeloid neoplasms-driver genes, making it difficult to link spliceosome mutations with downstream pathways. This prompted us to perform RNA-Seq on a cohort of 24 MDS bone marrow samples harboring solely spliceosome mutations (*SF3B1*, *SRSF2* P95 or frameshift deletion, *U2AF1* S34 or Q157 hotspot mutations, and *ZRSR2* truncating alterations) and devoid of other frequently co-occurring mutations, and 8 samples without spliceosome mutations as control. Analysis of splicing changes found overall low overlap of mis-spliced events between different splicing factor mutant groups, in agreement with earlier studies (Fig. 8). Still, splicing of 115 genes including disease related genes, affected two or more groups. For example, we noted increased inclusion of an *EZH2* exon harboring a premature stop codon ('poison' exon) in *SRSF2* and *U2AF1* mutated samples, similar to previous reports. Unique to our cohort, several *SF3B1* mutant MDS samples also showed increased usage of the same *EZH2* exon. qPCR analysis verified increased expression of *EZH2* transcripts bearing the 'poison' cassette exon, along with downregulation of canonical *EZH2* transcripts. As previously reported, some mutations affecting the same splicing factor gene resulted in non-overlapping splicing changes. Particularly, *U2AF1* S34 and Q157 mutations altered splicing of distinct sets of introns, with only one shared event. We observed that the splice variant of XBP1 (sXBP1) occurred at higher levels in *U2AF1* Q157 and *SRSF2* P95 samples but not in *U2AF1* S34 samples. These findings suggest increased endoplasmic reticulum stress and activation of unfolded protein response in specific mutant MDS. Among the different groups, *ZRSR2* mutant samples shared the largest number of events with *SRSF2* P95 samples. Interesting common targets include MAPK8, and the deubiquitinases USP10 and USP21. Initial pathways analysis showed that despite largely non-overlapping mis-spliced events, the genes affected by altered splicing in different mutant groups converge into common biological pathways (e.g. RNA splicing and transport, and various signaling cascades). Results of this work were recently published (**Madan et al, J Hematol. 2020**).

Drugs that kill *ZRSR2* mutated cells.

Recent studies revealed that cells bearing common gain-of-function splicing factor mutations (*SF3B1*, *U2AF1*, *SRSF2*) are preferentially sensitive to pharmacological modulation of splicing. Two approaches targeting splicing being tested clinically are: 1) splicing modulator drugs that target splicing factor *3B1* (e.g. E7107, H3B-8800), 2) sulfonamide drugs that degrade the auxiliary splicing factor RBM39 (e.g. E7070, E7820).

To identify additional functional targets that can be therapeutically targeted in cells with either *ZRSR2* mutations or mutations in other splining factor genes, synthetic lethal screens were performed in collaboration with Prof. J. Tyner (Oregon), using TF1 and K562 isogenic cells ± shZRSR2. For these experiments we used two parallel screening platforms: 1) A library of siRNAs directed against the entire tyrosine kinome (91 family members) as well as N-RAS, K-RAS, and control scrambled siRNAs, 2) a small-molecule panel (~130 drugs) of FDA-approved or late-stage development compounds. The screens identified several potentially interesting sensitivities in *ZRSR2* knockdown cell lines. For example, knockdown cells exhibited increased sensitivity to siRNA targeting ERBB3/4, and to inhibitors of the MAPK pathway (RAF265, an inhibitor of WT and oncogenic RAF, and CI-1040, a MEK1/2 inhibitor) (Fig. 9). Encouragingly, pathway analysis of mis-spliced genes in association with *ZRSR2* mutations in MDS patients showed enrichment of genes in the MAPK and ERBB signaling pathways. Of note, an inherited germline *ERBB3* variant is associated with a predisposition to erythroid MDS. Our results suggest strong dependencies of *ZRSR2*-deficient cells on MAPK and ERBB pathways for their viability; and, importantly, MAPK and ERBB inhibitors may be effective in patient with *ZRSR2* mutations.

Based on literature and our findings, we selected clinically relevant compounds (~10) and tested their effect on TF1 and K562 isogenic cells ± shZRSR2. Cells with silencing of ZRSR2 showed increased sensitivity for a number of drugs. For example, TF1-shZRSR2-cells are more sensitive to ATR inhibitor, VE-821 compared to TF1-shCont-cells (Fig. 10).

4. IMPACT

- **Impact on the development of the principal discipline(s) of the project**

Our studies help clarify the mechanistic basis for the pervasive nature of splicing disruption in MDS, leading to an overall better understanding of the disease. Specifically, this project filled significant gaps in the research area of splicing dysregulation in cancer by: 1) developing tools to study U12-type intron mis-splicing in MDS, 2) improving understanding of the molecular pathways linking impaired splicing and myeloid transformation, and 3) identifying potential therapeutic targets and biomarkers to predict response to treatments. The proposed work has direct translational implications by proposing drugs and/or combinations that will selectively kill *ZRSR2*-mutated cells. Because dysregulation of splicing is wide spared in cancer, our study has broad applicability to cancer research and treatment.

- **Impact on other disciplines**

Nothing to Report

- **Impact on technology transfer**

Nothing to Report

- **Impact on society beyond science and technology?**

Knowledge gained from this study may be harnessed to develop rational, effective treatments for MDS patients with splicing mutations.

5. CHANGES/PROBLEMS

- **Changes that had a significant impact on expenditures**

Nothing to Report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.**

Nothing to Report

6. PRODUCTS

Nothing to Report

7. FIGURES

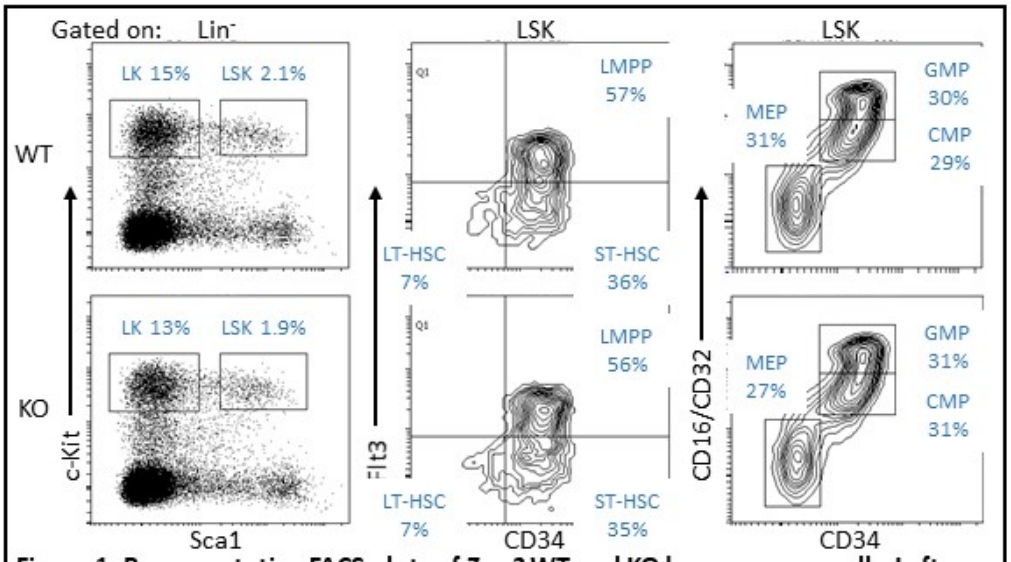

Figure 1. Representative FACS plots of *Zrsr2* WT and KO bone marrow cells. Left: Lineage$^-$ cells gated on c-Kit and Sca-1 to distinguish LK (Lin$^-$Sca1$^-$Kit$^+$) and LSK (Lin$^-$ Sca1$^+$Kit$^+$) cells. **Middle:** LSK cells co-stained with Flt3 and CD34 to distinguish LMPP (lymphoid-primed multipotent progenitors), LT-HSC (long-term stem cells) and ST-HSC (short term HSC). **Right:** LSK cells co-stained with CD16/ CD32 and CD34 to distinguish MEP (megakaryocyte-erythrocyte progenitors), GMP (granulocyte-monocyte progenitors), and CMP.(common myeloid progenitors).

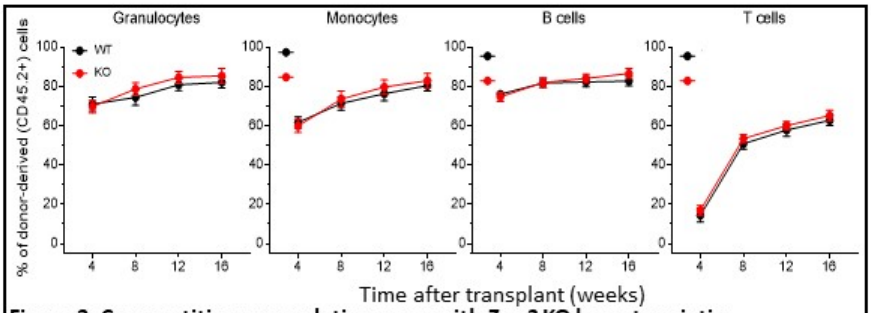

Figure 2. Ccompetitive repopulation assay with *Zrsr2* KO hematopoietic progenitors. 2x10^3 LSK (Lin$^-$Sca1$^+$Kit$^+$) cells (CD45.2) from either WT or KO mice were mixed with 5x10^5 LSK WT competitor cells (CD45.1) and injected into lethally irradiated congenic (CD45.1) mice. Graphs show the percentage of donor chimerism (CD45.2$^+$) measured in peripheral blood of recipients.

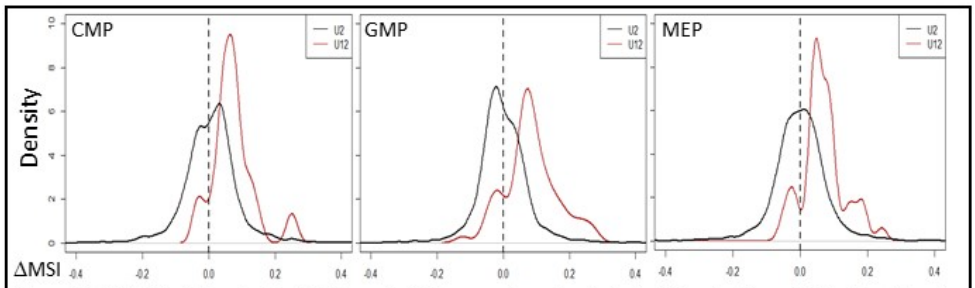

Figure 3. Global splicing in *Zrsr2* KO myeloid linages. Density plots depict retention of U2- (black) and U12-type introns (red) in *Zrsr2* KO CMP (common myeloid progenitors), GMP (granulocyte-monocyte progenitor) and MEP (megakaryocyte -erythrocyte progenitors) cells compared with WT cells. RNA-Seq was performed on sorted cells from three KO and WT littermates. ΔMSI; difference in Mis-Splicing Index.

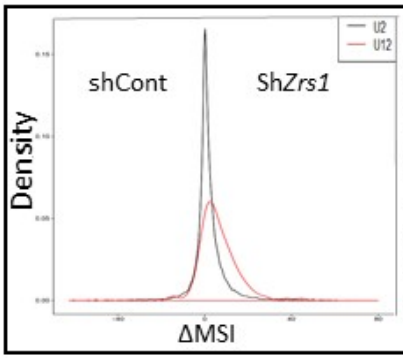

Figure 4. Density plots depict retention of U2- (black) and U12-type introns (red) in *Zrsr2* KO Lin-Kit$^+$ cells transfected with either shCont-GFP or sh*Zrsr1*-RFP. RNA-Seq was performed on GFP/RFP sorted cells. ΔMSI; difference in Mis-splicing Index.

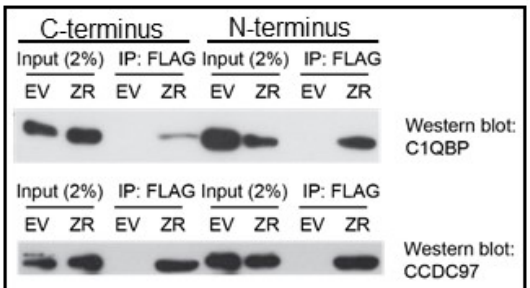

Figure 5. ZRSR2 interacts with C1QBP and CCDC97. C-terminus and N-terminus FLAG - ZRSR2 proteins were overexpressed in 293T cells. Proteins were immunoprecipitated with FLAG antibody followed by western blotting with either C1QBP or CCDC97 antibodies.

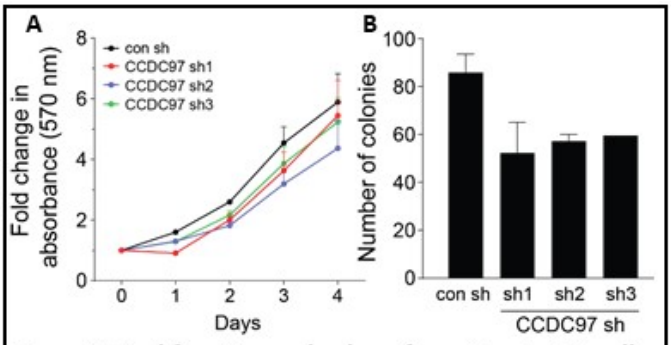

Figure 6. Proliferation and colony-formation in TF1 cells with knockdown of CCDC97. (**A**) MTT proliferation assays. (**B**) Methylcellulose colony assays.

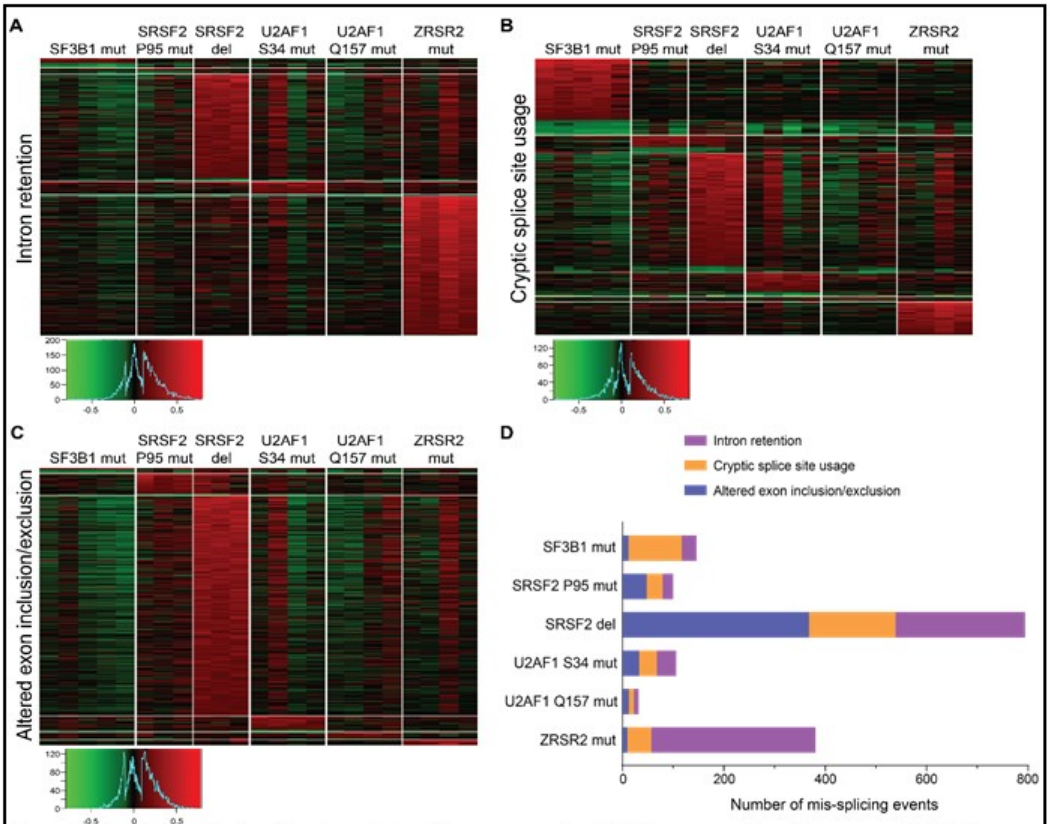

Figure 8. Clustering of mis-spliced events in spliceosome mutant MDS compared to spliceosome WT MDS. (**A-C**) Heat maps show cluster of significantly mis-spliced events for each mutant group. Splicing events were categorized as intron retention (**A**), cryptic splice site usage (**B**) and altered exon inclusion/exclusion (**C**). (**D**) Number and type of significant splicing changes identified in each group bearing a spliceosome mutation.

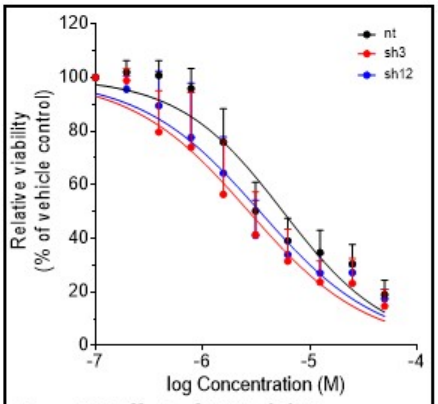

Figure 10. Effect of ATR inhibition on viability of ZRSR2 knockdown TF1 cells. Cells were treated with increasing concentration of ATR inhibitor VE-821. MTT proliferation assays were performed on day 4.

8. PUBLICATIONS

1. **Distinct and convergent consequences of splice factor mutations in myelodysplastic syndromes.** Madan V, Li J, Zhou S, Teoh WW, Han L, Meggendorfer M, Malcovati L, Cazzola M, Ogawa S, Haferlach T, Yang H, Koeffler HP. Am J Hematol. 2020 Feb;95(2):133-143. doi: 10.1002/ajh.25673. Epub 2019 Nov 18. PMID: 31680297

Abstract: Myelodysplastic syndromes (MDS) are characterized by recurrent somatic alterations often affecting components of RNA splicing machinery. Mutations of splice factors SF3B1, SRSF2, ZRSR2 and U2AF1 occur in >50% of MDS. To assess the impact of spliceosome mutations on splicing and to identify common pathways/genes affected by distinct mutations, we performed RNA-sequencing of MDS bone marrow samples harboring spliceosome mutations (including hotspot alterations of SF3B1, SRSF2 and U2AF1; small deletions of SRSF2 and truncating mutations of ZRSR2), and devoid of other common co-occurring mutations. We uncover the landscape of splicing alterations in each splice factor mutant MDS and demonstrate that small deletions in SRSF2 cause highest number of splicing alterations compared with other spliceosome mutations. Although the mis-spliced events observed in different splice factor mutations were largely non-overlapping, a subset of genes, including EZH2, were aberrantly spliced in multiple mutant groups. We also verified aberrant splicing of key genes USP9X, USP24 (deubiquitinating enzymes), LUC7L2 (splice factor) and EED (PRC2 component) in MDS harboring small deletions of SRSF2. Pathway analysis revealed that mis-spliced genes in different mutant groups were enriched in RNA splicing and transport as well as several signaling cascades, suggesting converging biological consequences downstream of distinct spliceosome mutations. Our study reveals splicing signatures of each splice factor mutation and identifies shared and distinct sets of mis-spliced genes and affected biological processes in different spliceosome mutant MDS.

2. **Identification of somatic alterations in lipoma using whole exome sequencing.** Kanojia D, Dakle P, Mayakonda A, Parameswaran R, Puhaindran ME, Min VLK, Madan V, Koeffler HP. Sci Rep. 2019 Oct 7;9(1):14370. doi: 10.1038/s41598-019-50805-w.PMID: 31591430

Abstract: Lipomas are benign fatty tumors with a high prevalence rate, mostly found in adults but have a good prognosis. Until now, reason for lipoma occurrence not been identified. We performed whole exome sequencing to define the mutational spectrum in ten lipoma patients along with their matching control samples. We presented genomic insight into the development of lipomas, the most common benign tumor of soft tissue. Our analysis identified 412 somatic variants including missense mutations, splice site variants, frameshift indels, and stop gain/lost. Copy number variation analysis highlighted minor aberrations in patients. Kinase genes and transcriptions factors were among the validated mutated genes critical for cell proliferation and survival. Pathway analysis revealed enrichment of calcium, Wnt and phospholipase D signaling in patients. In conclusion, whole exome sequencing in lipomas identified mutations in genes with a possible role in development and progression of lipomas.

3. **Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome.**Madan V, Kanojia D, Li J, Okamoto R, Sato-Otsubo A, Kohlmann A, Sanada M, Grossmann V, Sundaresan J, Shiraishi Y, Miyano S, Thol F, Ganser A, Yang H, Haferlach T, Ogawa S, Koeffler HP. Nat Commun. 2015 Jan 14;6:6042. doi: 10.1038/ncomms7042.PMID: 25586593

Abstract: Somatic mutations in the spliceosome gene ZRSR2-located on the X chromosome-are associated with myelodysplastic syndrome (MDS). ZRSR2 is involved in the recognition of 3'-splice site during the early stages of spliceosome assembly; however, its precise role in RNA splicing has remained unclear. Here we characterize ZRSR2 as an essential component of the minor spliceosome (U12 dependent) assembly. shRNA-mediated knockdown of ZRSR2 leads to impaired splicing of the U12-type introns and RNA-sequencing of MDS bone marrow reveals that loss of ZRSR2 activity causes increased mis-splicing. These splicing defects

involve retention of the U12-type introns, while splicing of the U2-type introns remain mostly unaffected. ZRSR2-deficient cells also exhibit reduced proliferation potential and distinct alterations in myeloid and erythroid differentiation in vitro. These data identify a specific role for ZRSR2 in RNA splicing and highlight dysregulated splicing of U12-type introns as a characteristic feature of ZRSR2 mutations in MDS.