Myeloid Malignancies Associated with Spliceosome Mutation

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Matthew Walter, M.D.
Washington University School of Medicine
St. Louis, Missouri, USA
DISCLOSURE

• I have no relevant financial relationships to disclose.
What spliceosome gene is most commonly mutated in MDS patients with ringed sideroblasts?

- A. SF3B1
- B. SRSF2
- C. U2AF1
- D. ZRSR2
Splicing Factor Gene Mutations in MDS

Myelodysplastic syndromes = 51% with SF mutation (n=2400)

1) Mutually exclusive mutations
2) Heterozygous mutations
3) Founding clone mutations

Therapeutic implications

Splicing Factor Gene Mutations in Other Cancers

Spliceosome Gene Mutations Do Not Define MDS

CCUS Evolution to MDS/AML

- **154 cytopenic patients** without myeloid neoplasm
- **36%** (56/154) had 1 or more mutations in 40 genes = CCUS
- Highest progression risk = **SF3B1, SRSF2, U2AF1** or **DNMT3A, TET2, or ASXL1 + other**

Malcovati, *Blood*, 2017

(hazard ratio [HR] = 13.9; P < 0.001)
Do spliceosome gene mutations alter pre-mRNA splicing?
Sequence-Specific Motifs in Alternatively Spliced RNA


modified from Lindsley & Ebert, Review in Advance, 2012
Do spliceosome gene mutations contribute to disease pathogenesis?
Modeling Hotspot Mutations


[Diagram showing U2AF1, SRSF2, and SF3B1 with hotspot mutations labeled.]
## Mutant Mice have Altered Hematopoiesis

<table>
<thead>
<tr>
<th>Gene</th>
<th>WBC</th>
<th>RBC</th>
<th>Comp. Repop.</th>
<th>Altered RNA-splicing</th>
<th>MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>U2AF1(S34F)-Tg</td>
<td>↓</td>
<td>NC</td>
<td>↓</td>
<td>Exon skip “T”AG</td>
<td>No</td>
</tr>
<tr>
<td>U2af1(S34F)-KI</td>
<td>↓</td>
<td>↓ Hgb., MCV</td>
<td>↓</td>
<td>Exon skip “T”AG</td>
<td>No</td>
</tr>
<tr>
<td>Srsf2(P95H)-KI</td>
<td>↓</td>
<td>↓ Hgb., MCV</td>
<td>↓</td>
<td>Altered affinity for ESE motif</td>
<td>MDS-like</td>
</tr>
<tr>
<td>Sf3b1(K700E)-KI</td>
<td>NC</td>
<td>↓ Hgb., MCV</td>
<td>↓</td>
<td>Cryptic 3’ SS usage</td>
<td>RA-like</td>
</tr>
</tbody>
</table>

Tg = transgenic; KI = knock-in; NC = no change

Do splicing alterations contribute to disease pathogenesis \textit{in vivo}?
H2AFY1.1 Expression is Reduced in U2AF1(S34F) MDS

U2AF1(S34F) → {↑ Myeloid progenitors, ↓ Monocytes, ↓ B-cells}

H2AFY1.1 expression in MDS

Shirai, Cancer Cell, 2015
Reduced H2AFY1.1 Expression Alters B cells

Add-back H2AFY1.1

1. H2afy null mice
2. U2AF1(S34F) mice

Kim, unpublished
Can we target spliceosome mutant cells?

Why are spliceosome mutations.....

1) Mutually exclusive?
2) Heterozygous?

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<tr>
<th>SF3B1</th>
<th>SRSF2</th>
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</tbody>
</table>

Obeng, *Cancer Cell* (2016)  
Fei, *PNAS* (2018)
1) Co-Expression of Two Spliceosome Gene Mutations is Not Tolerated

Lee, Cancer Cell (2018) Graubert, Fei, Varmus, unpublished
2) Spliceosome Mutant Cells Require Expression of the Wild-Type Allele

Srsf2(P95H)

U2af1(S34F)

Lee, Cancer Cell (2018)

Wadugu, Fei, Varmus, Walter, unpublished
• **Hypothesis**: Cells harboring a spliceosome gene mutation require the residual function of normal splicing factors
Splicing modulators that bind SF3B1

Inhibition of SR protein phosphorylation or methylation

Approaches to Modulate RNA Splicing

Saez, Blood (2017)
Splicing Modulators that Bind SF3B1

Pladienolide B: $R = \text{CH}_3$, $R'' = \text{H}$
E7107: $R = 4$-cycloheptylpiperazin-1-yl, $R'' = \text{OH}$

H3B-8800 (Phase I = NCT02841540)

FR901464: $R' = \text{H}$
Spliceostatin: $R' = \text{Me}$

Sudemycin D6: $X = \text{CH}_2$, $R = \text{NHCH}_3$

SF3B1 & PHF5A Resistance Mutations

E7107
Herboxidiene
Sudemycin D6

Shirai, unpublished

Herboxidiene
Sudemycin D6

Teng, Nat Comm (2017)

H3B-8800

Seiler, Nat Med (2018)
R-Loops Create a Vulnerability in Mutant Cells

Summary

1) Spliceosome gene mutations are typically in the founding clone in MDS and have prognostic significance

2) Spliceosome mutant cells are dependent on the residual function of normal splicing factors for survival

3) Cells harboring a spliceosome gene mutation have increased sensitivity to splicing modulator drugs and ATR inhibition
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