SF3B1 mutations induce oncogenic IRAK4 isoforms and activate targetable innate immune pathways in MDS and AML

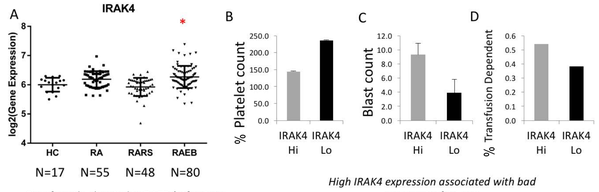
Gaurav S. Choudhary[1]*, Andrea Pellagatti[2]*, Molly A Smith[3], Tushar D Bhagat[1], Shanisha Gordon-Mitchell[1], Sanjay Pandey[1], Nishi Shah[1], Srinivas Aluri[1], Robert Booher[4], Murali Ramachandra[5], Maria Elena Samson[4], Kith Pradhan[1], Teresa V. Bowman[1], Manoj Pillai[6], Chandan Guha[1], Amittha Wickrema[7], Britta Will[1], Aditi Shastri[1], Ulrich Steidl[1], Jacqueline Boultwood[2]*, Daniel T. Starczynowski[3]*, Amit Verma[1]*

[1]Albert Einstein College of Medicine, Bronx, NY; [2]University of Oxford, UK; [3]Cincinnati Children's Hospital Medical Center, Cincinnati OH; [4]Curis, Inc., MA; [5]Aurigene, India; [6]Yale University, CT; [7]University of Chicago, Chicago, IL.

Abstract

SF3B1 mutations are the most frequently occurring splicing factor mutations in MDS and AML, however the misspliced genes that contribute the malignant state in SF3B mutant MDS or AML remains unclear. We determined that SF3B1 mutant cases of MDS express a longer, active isoform of interleukin 1 receptor associated kinase (IRAK4). IRAK4 is a serine/threonine kinase that is downstream of toll-like receptor (TLR) signaling and leads to activation of oncogenic signaling states, including NF-kB and MAPK. Examination of IRAK4 by RNA sequencing showed that normal cells predominantly express small IRAK4 isoforms resulting from exclusion of the part of exon 6. These isoforms are targeted for proteosomal degradation leading to diminished IRAK4 expression and activation in normal cells. In contrast, a large proportion of MDS/AML samples with SF3B1 mutation show increased expression of an IRAK4 isoform that retains full exon 6, encoding the full-length protein (IRAK4-Long). Consequently, we show that expression of mutant SF3B1-K700E in leukemic cells is associated with increased NF-kB activity, suggesting that mutations in SF3B1 instruct expression of IRAK4 RNA isoforms with maximal functional potential. Furthermore, SF3B1 mutant MDS and AML cells exhibited a block in hematopoietic differentiation in clonogenic assays. This differentiation block was ameliorated with pharmacologic inhibition of IRAK4 with CA-4948, a potent oral clinically useful small-molecule inhibitor of IRAK4. CA-4948 blocked TLR-stimulated cytokine release in various cell models and also led to decreased leukemic burden in mice xenografted with SF3B1 mutant MDS/AML cells. Finally, we determined that SF3B1 mutation induced IRAK4 activation led to TRAF6 mediated K63 ubiquitination of critical cell cycle and regulatory proteins directly implicated in oncogenesis. We had recently shown that U2AF1 mutations can lead to IRAK4 activation via retention of exon 4 (Smith et al, Nat Cell Bio, 2019). Our data now demonstrate that SF3B1 leads to overactivation of IRAK4 via retention of a different exon (exon 6), thus reinforcing that IRAK/TRAF6 activation is a common downstream oncogenic pathway in splicing factor mutated MDS/AML. Taken together, in this study, we find that mutations in SF3B1 induce expression of therapeutically targetable "active" IRAK4 isoforms and provide a genetic link between a spliceosome mutation and oncogenic innate immune signaling in MDS and AML.

IRAK4 is elevated in MDS



Significantly elevated in RAEB (refractory anemia with excess blasts)- worst subgroup

High IRAK4 expression associated with bad prognostic features

IRAK4 Expression	Median Survival (y)
High	2.6
low	5.2

High IRAK4 expression associated with worse overall survival

Figure 1: IRAK4 is overexpressed in MDS stem and progenitor cells and is associated with worse prognosis in MDS : A: IRAK4 expression in 183 MDS bone marrow derived CD34+ cells and 17 healthy control CD34+ cells is shown. IRAK4 is overexpressed in MDS patients with Refractory Anemia with Excess of Blasts (RAEB, TTest, P Value<0.05).RA (Refractory anemia), RARS (Refractory anemia with ringed sideroblasts) and RAEB (Refractory anemia with excess of blasts).
B-D: Mean percentage of platelets, leukemic blast cells and rate of transfusion dependence in patients with low and high IRAK4 expression is shown (Ttest, P Value <0.05).
E: Kaplan Meir curves for patients with MDS (N=183)(E) are shown. Log rank P value was < 0.05 with worse survival for samples with higher expression of IRAK4.

IRAK4-L expression is associated with SF3B1 mutations in MDS/AML

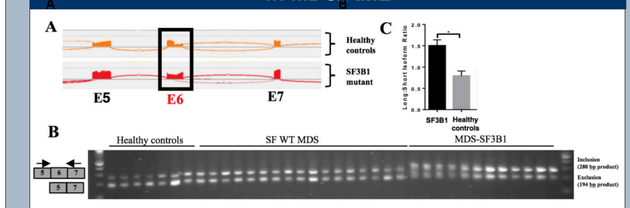


Figure 2: IRAK4-L expression is associated with SF3B1 mutations in MDS/AML: (A) Sashimi plots representing IRAK4 exon 6 inclusion or exclusion in healthy CD34+ cells, CD34+ cells isolated from patients with mutation in SF3B1 based on RNA-sequencing junction reads. Patients with SF3B1 mutation expressed IRAK4-L, while some MDS patients without splicing factor mutations expressed IRAK4-L (MDS-SF wild-type) (B) RT-PCR analysis of healthy CD34+ cells (n = 7 samples), CD34+ cells isolated from MDS patients with no splicing factor mutations (MDS-SFWT; n = 17 samples), and from patients with mutation in SF3B1 (MDS-SF3B1mut; n = 12 samples) using primers flanking IRAK4 exon 6. (C) Graph shows the ratio of IRAK4-Long :IRAK4 short isoform which is more in SF3B1 patients as compared to that of healthy controls.

IRAK4 isoforms and their activation in innate immune signaling in MDS/AML

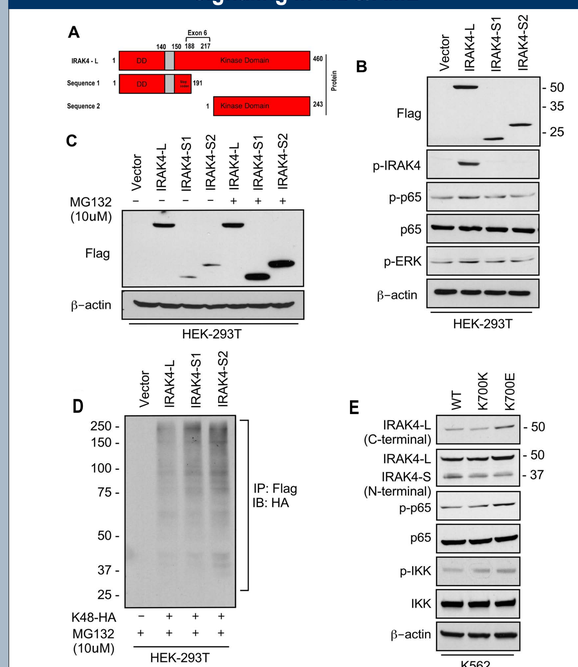


Figure 3: Oncogenic IRAK4 isoforms in SF3B1 mutant cells results in maximal activation of innate immune signaling : A. IRAK4 isoforms in MDS/AML. IRAK4 isoforms plasmids were expressed in HEK-293T cells. B. Western blot analysis of indicated proteins in down-stream NF-kB and MAPK pathway. C. Transfected cells were incubated with MG132 for 9 hrs. Western blot was performed and probed with Flag ab. D. Ubiquitination of short isoforms of IRAK4 was determined in HEK-293T cells transfected with IRAK4 and Lys 48-HA plasmids. immunoprecipitating was performed with Flag antibody and immunoblotting with HA. E. Immunoblot analysis for indicated proteins in Wild type and SF3B1-K700E K562 cells

IRAK4 inhibitor leads to reduced viability in leukemic stem and progenitors

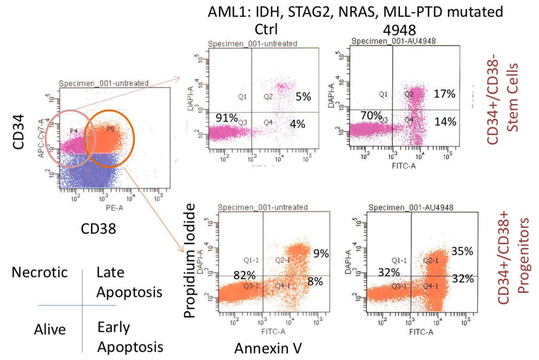
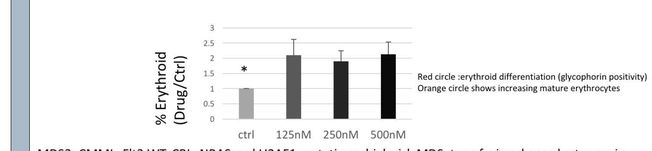


Figure 4: IRAK4 inhibitor reduces viability in leukemic CD34+/CD38- HSCs : AML sample was treated with IRAK4 inhibitor and viability was assessed in the CD34+/CD38- HSC and CD34+/CD38+ Progenitor compartments. Viability in AML HSCs and Progenitors was significantly decreased after IRAK4 inhibition.

IRAK4 inhibitor increases erythroid differentiation from primary MDS/AML HSPCs



MDS3: CMML, Flt3 WT, CBL, NRAS and U2AF1 mutation+, high risk MDS, transfusion dependent anemia

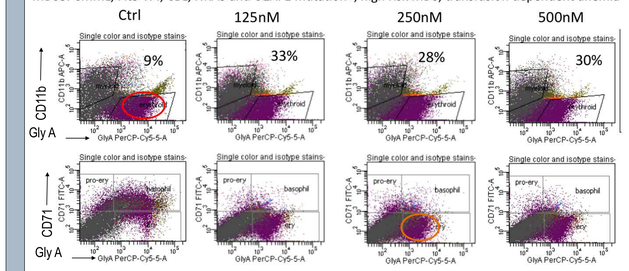


Figure 5: IRAK4 inhibitor leads to enhanced erythroid differentiation in MDS primary samples: MDS stem and progenitor cells were grown in methylcellulose for 14 days with and without IRAK4 inhibitor. Colonies were analyzed for differentiation by multiparameter flow cytometry. Increased erythroid differentiation was seen (higher Glycophorin A positivity) after IRAK4 inhibition. Increased maturation of erythroid cells was also demonstrated (increased percentages of late erythroid) after IRAK4 inhibition

IRAK4 inhibition increases myeloid differentiation from primary MDS/AML HSPCs

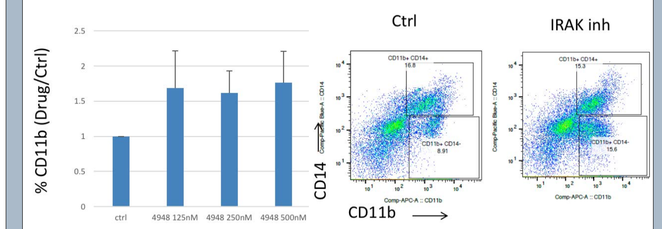


Figure 6: IRAK4 inhibition leads to enhanced myeloid differentiation in MDS primary samples: MDS stem and progenitor cells were grown in methylcellulose for 14 days with siCTL and siIRAK4. Colonies were picked and analyzed for differentiation by multiparameter flow cytometry. Increased myeloid differentiation was seen (higher CD11b positivity) after IRAK4 inhibition. Representative example is shown.

IRAK4 inhibitor causes decreased leukemic engraftment and increased differentiation in vivo in PDX models

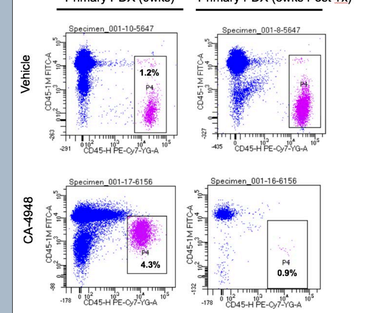


Figure 8: IRAK4 inhibitor leads to decreased leukemic engraftment in PDX models: Xenografts in immunodeficient NSG mice were established with primary AML/MDS samples. Mice were treated with IRAK4 inhibitor or control and sacrificed. Mice treated with IRAK4 inhibitor demonstrated reduced human leukemic disease burden by flow (A).

Conclusions

1. Increased IRAK4 expression in seen in human MDS/AML stem and progenitor samples.
2. IRAK4 isoforms are differentially expressed in MDS/AML samples
3. IRAK4-L isoform causes maximal activation in innate immune signaling. The shoter IRAK4 isoforms in WT SF3B1 are targeted for proteasomal degradation.
4. IRAK4 inhibitor caused increased differentiation from MDS/AML samples in vitro
5. IRAK4 inhibitor decreases disease burden in leukemic xenografts
6. IRAK4 inhibitor results in decreased engraftments in primary human AML/MDS PDX models