Emerging Immune-related therapies in MDS/AML

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I have no relevant financial relationships to disclose
Overview

• Genetic and molecular mechanisms contributing to dysregulation of immune pathways in MDS.

• Epistasis between miR-146a and TIFAB, two genes in del(5q) MDS.

• Proof-of-concept and pre-clinical data on emerging immune-related therapies in MDS.
Aberrant immune function is a hallmark of MDS

- Increased inflammatory cytokines and chemokines:
  - IL-1, IL-6, TNFα, IFNγ, TGFβ
- Overexpression of immune receptors and ligands:
  - TLR1/2/4/6, Alarmins/DAMPs (S100A8/A9, circulating DNA)
- Altered immune cell populations:
  - Regulatory T cells, Myeloid-derived suppressor cells
- Associated with autoimmune and inflammation disorders:
  - Rheumatoid arthritis, Inflammatory bowel disease.
Innate Immune System: Toll-like receptor (TLR) signaling in immune effector cells

- Pathogens
- DAMPs
- TLR

- MyD88
- IRAK1
- IRAK4
- TRAF6
- TAB2
- TAB3
- TAK1
- JNK
- p38
- miR-146a
- A20

- IKK complex
- IκB
- p50
- RelA

- Cytokines
- Chemokines
- Cell proliferation
- Differentiation
- Anti-microbials

- Time Response
- Pro-inflammatory
- Anti-microbial
- Tolerance
- Proliferation
- Differentiation
Multiple genetic and molecular mechanisms contribute to hyperactivation of TLR signaling in MDS HSPC

- Increased ligands (S100A8/9)
- Activating mutations & overexpression of TLRs
- Overexpression & activation of IRAKs and TRAF6
- Deletion of negative regulators (del5q)
- Ineffective hematopoiesis and MDS

- miR-146
- TIFAB
- TRAF6
- IRAK4
- IRAK1
- TLR
- NF-κB
- MAPK

References:
Immune signaling contributes to hematopoietic defects and MDS by cell extrinsic and intrinsic mechanisms.

Reactive/Cell Extrinsic

HSPC Intrinsic

Why do MDS cells exhibit increased TLR signaling and what are the hematopoietic consequences?

- Increased ligands (S100A8/9)
- Activating mutations & overexpression of TLRs
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- Deletion of negative regulators (del5q)
- Ineffective hematopoiesis and MDS
miR-146a and TIFAB, two del(5q) MDS genes, restrict the TLR pathway in HSPC

Identification of miR-145 and miR-146a as mediators of the 5q–syndrome phenotype

Daniel T Starczynowski1,2, Florian Kuchenbauer1, Bob Argiropoulos1, Sandy Sung1, Ryan Morin1, Andrew Muranyi1, Martin Hirsi1, Donna Hogge1, Marco Marra1, Richard A Wells5, Rena Buckstein3, Wan Lam1,2, R Keith Humphries1,4 & Aly Karsan1,2

miR-146a deficiency:
- Transient myeloproliferation/BM failure/MDS
- Altered hematopoietic stem and progenitor cell function
- Increased TRAF6 and IRAK1 mRNA stability
- Sensitive to TLR activation

TIFAB deficiency:
- Peripheral blood cytopenia
- Altered hematopoietic stem and progenitor cell function
- Derepression of TRAF6 protein
- Sensitive to TLR activation

Cell Reports
Myeloid Malignancies with Chromosome 5q Deletions Acquire a Dependency on an Intrachromosomal NF-κB Gene Network

Jing Fang1, Brenden Barker1, Lyndsey Bolanos1, Xiaona Liu1, Andres Jerez2, Hideki Makishima2, Susanne Christie3, Xiaoting Chen4, Dinesh S. Rao1, H. Leighton Grimes2, Kakajan Komurov5, Matthew T. Weirauch6,7, Jose A. Canelas2,8, Jaroslav P. Maciejewski9,10 & Daniel T. Starczynowski1,2

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*Correspondence: daniel.starczynowski@cchmc.org

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Reviewed in Zhao and Starczynowski, Frontiers (2014)
miR-146a and TIFAB, neighboring 5q genes, cooperate to regulate TRAF6 and IRAK1/4

Varney et al., Leukemia (2016)
Deletion of miR-146 and TIFAB increases the severity of hematologic defects

Varney et al., Leukemia (2016)
TRAF6 overexpression results in differential sensitivity to TLR agonists

Fang et al., Nature Immunology (in press); Muto et al., unpublished
**Summary I: Regulation of TLR/TRAF6 by miR-146 and TIFAB, two del(5q) MDS genes**

- Cooperating genetic events in del(5q) MDS activate the TLR/TRAF6 pathway.
  - miR-146a = TRAF6 mRNA
  - TIFAB = TRAF6 protein

- Combined deletion of miR-146a and TIFAB synergistically regulates gene networks in HSPC.
  - Interferon, EGFR, immune regulation, and HSC/myeloid differentiation
  - Increased sensitivity to TLR ligands

- Combined deletion of miR-146a and TIFAB (via TRAF6 and IRAK1/4 activation) impacts HSPC function and severity of hematologic defects.
Can the TLR signaling complex be targeted in MDS?

Rhyasen et al., Cancer Cell (2013)
IRAK1 is activated in MDS

Rhyasen et al., Cancer Cell (2013)
Knockdown of IRAK1 or IRAK4 impairs MDS/AML HSPC function \textit{in vitro}

Rhyasen et al., Cancer Cell (2013)
Rhyasen et al., Exp Hem (2014)
Knockdown of IRAK1 impairs MDS HSPC function \textit{in vivo}

MDSL \rightarrow \text{inducible} \rightarrow \text{shIRAK1} \rightarrow \text{NSG} \rightarrow \text{-DOX} \rightarrow \text{+DOX}

\text{Percent Survival}

\begin{tabular}{c c c}
0 & 1 & 2 \\
\text{DOX (\mu g/ml)} & IRAK1 & GAPDH \\
\end{tabular}

\text{Rhyasen et al., Cancer Cell (2013)}
Pharmacological inhibition of IRAK1/4

IRAK-Inh (acyl-2-aminobenzamidazole)

Rhyasen et al., Cancer Cell (2013)
Pharmacological inhibition of IRAK1/4 in primary MDS/AML

MDS-02

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AML-03

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Rhyasen et al., Cancer Cell (2013)
IRAK-Inh suppresses MDS/AML cell lines *in vitro*

Rhyasen et al., Cancer Cell (2013)
IRAK-Inh is effective at targeting primary MDS cells, but spares normal BM HSPC.
Pre-treatment of MDSL cells with IRAK-Inh suppresses disease *in vivo*

MDSL

\[+/- \text{ IRAK-Inh}\]

24 hr in vitro culture

NSG/NSGS

Overall survival

BM/PB analysis

Rhyasen et al., Cancer Cell (2013)
In vivo IRAK-Inh treatment improves anemia and diminishes MDSL engraftment

MDSL → NSG
  ↓ 2 weeks
  ↓ +/-IRAK-Inh (3x per week)
  ↓ BM/PB analysis

Rhyasen et al., Cancer Cell (2013)
Summary II: IRAK1/4 as a target for MDS and AML

- IRAK1 and IRAK4 is hyperactivated and required for maintaining the leukemic state of MDS and AML cells via TRAF6 signaling.

- IRAK-Inh targets MDS-propagating cells:
  - increased apoptosis
  - reduced progenitor function
  - no effect on normal CD34+ cells
  - not sufficient to target AML-propagating cells

- Dire need of compounds targeting IRAK1/4 with drug-like properties and clinical potential.
Alternative and emerging immune-related targeting approaches for MDS

1. S100A9 chimeric decoy receptor (CD33-IgG1)
2. TLR2 neutralization IgG4
3. IRAK1/4 inhibitors
4. Downstream effectors (p38 inhibitors)
Conclusions: Chronic innate immune signaling is an important mechanism and druggable target in MDS

- Chronic immune signaling contributes to cell-intrinsic and systemic hematopoietic defects that contribute to the pathogenesis of MDS.
- Combined deletion of TIFAB and miR-146a, two del(5q) MDS genes, enhance TRAF6 signaling and contribute to hematopoietic defects.
- TRAF6 overexpression is sufficient to induce hematopoietic defects and features of MDS.
- Targeting the TLR/IRAK/TRAF6 signalosome may be an effective therapeutic strategy against MDS-propagating cells.
Final Remark

TLR/TRAF6 induced MDS/AML is a two-step process:

- one step provides the normal cell with the new ability to activate TLR/TRAF6 as a consequence of aberrant epigenetic regulation or deletion of negative regulators;

- the second step involves progression to a malignant state through the constitutive activation of oncogenic pathways mediated by TLR/TRAF6 signaling.
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