Immune microenvironment dysfunctions enable malignification at the onset of MDS


Background

- **Myelodysplastic syndromes (MDS):** clonal stem cell malignancies
- **Standard therapy not curative, transient responses, poor prognosis**
- **Are prevention or early intervention possible in MDS?**
- **Clonal cytopenias of undetermined significance (CCUS):** aging-related premalignant state, low-grade clonal hematologic disorders at high risk of progression to MDS and leukemia
- HSC-intrinsic alterations and extrinsic inflammatory factors cooperate to induce abnormal differentiation in CCUS (Ganan-Gomez et al. ASH Meeting 2021).

Aim

- Characterize early alterations in the bone marrow (BM) immune microenvironment that lead to the expansion of the MDS clone

Methods

- BM mononuclear cells (MNCs) from patients with CCUS and young and elderly healthy donors (HDs)
- Single-cell transcriptomics (scRNA-seq)
- *In vitro* functional assays
- Single-cell genomics/antigen expression analysis (Tapestri)

1. BM Innate Immune Cells Are Activated in CCUS Patients

Pathway analysis of significantly upregulated genes in CCUS

- Formation of ATP by chemiosmotic coupling
- Interferon alpha/beta signaling
- Neutrophil degranulation
- Mitochondrial translation initiation
- Cytokine Signaling in Immune system
- Membrane Trafficking
- Eukaryotic Translation Elongation
- Respiratory electron transport, ATP synthesis by chemiosmotic coupling
- Adaptive Immune System
- Parasite infection
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2. Inhibitory Crosstalk Predicted Between NK and CD8+ T Cells in CCUS

- NK cell activation by CD8+ T cells
  - HLA-E:CD94/NKG2C
  - CD58:CD2
  - FASLG:TNFRSF1A

- Immunosuppression of CD8+ T cells by NK cells
  - CD48:CD244
  - TGFB1:TGFBR1

3. CCUS NK/T Cells Are Terminally Differentiated toward Exhaustion

scRNA-seq in sorted CD3+ T and CD56+ NK cells

CytoTRACE differentiation scores

Exhaustion marker expression
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4. NK Cells from CCUS Patients Are Irreversibly Dysfunctional

In vitro cytolysis assays with leukemic cell lines

Post-7-day NK cell expansion

- K562 alone
- THP1 alone
- Young Healthy
- Old Healthy
- CCUS

Conclusions

- The innate immune repertoire is molecularly and functionally altered in patients with CCUS
- Immune evasion of premalignant clones may contribute to clonal evolution to MDS
- Rationale for adoptive NK cell therapy in patients with CCUS or low-burden MDS to prevent/arrest MDS progression

5. NK Cells Are Part of the Mutant CCUS Clone

Joint scDNA-seq and surface protein analysis (Tapestri)

CD8+ T

CD4+ T

T Prog

NK cells

B cells

cDC

pDC

CD16+ Mono

My/Mono

Post-7-day NK cell expansion

- K562 alone
- THP1 alone
- Young Healthy
- Old Healthy
- CCUS

% Normalized K562 counts

% Normalized THP1 counts

Time (h)

Time (h)