

Myelodysplastic syndromes - Section 14

The role of innate immunity in MDS pathogenesis

David A. Sallman, Alan List

Malignant Hematology Department, H. Lee Moffitt Cancer Center and Research Institute, Tampa, United States

Take home messages

- Innate immune activation, driven by MDSC expansion and/or mesenchymal niche cells, may be a disease initiating event in MDS.
- Cell-extrinsic and-intrinsic signals provoke NLRP3 inflammasome activation that directs an inflammatory cell death, termed pyroptosis, leading to the hallmark pathological features of MDS.
- Inflammasome activation has potential broad reaching importance as a diagnostic biomarker and therapeutic target in MDS patients.

Introduction

The clinical phenotype of myelodysplastic syndromes (MDS) is manifested by ineffective hematopoiesis, bone marrow dysplasia and a propensity for transformation to acute myeloid leukemia (AML). Comprehensive molecular interrogation of MDS via next-generation sequencing can identify somatic mutations in the vast majority of patients with critical diagnostic, prognostic and therapeutic implications. However, how mutations in a diverse array of functional classes (eg, spliceosome, epigenetic, transcription) can converge upon a common hematologic phenotype was unknown. Although aberrant innate immune signaling and a pro-inflammatory microenvironment have long been implicated in the pathogenesis of MDS, elucidation of the precise underlying mechanisms linking these inflammatory processes to the above clonal drivers has only recently been advanced. Central to this advancement was the identification of the danger-associated molecular pattern (DAMP) protein S100A9 that directs an inflammatory cell death, termed pyroptosis, via activation of the NLRP3 inflammasome. Furthermore, S100A9 release is integrally linked to the bone marrow microenvironment via expansion of myeloid derived suppressor cells (MDSCs) and mesenchymal niche cells. Additionally, cell-intrinsic events (ie, somatic gene mutations) directly drive S100A9 overexpression, providing a feed-forward mechanism to further amplify pyroptotic cell death. Notably, activation of the NLRP3 inflammasome complex

has also been identified as a diagnostic biomarker in MDS as well as a central process in the cardiovascular morbidity/mortality associated with age-related clonal hematopoiesis. Together, these advancements should ultimately translate to novel, disease modifying therapies (Fig. 1).

Current state of the art

Aberrant activation of the innate immune system has long been recognized in MDS, manifested by over expression and activation of Toll-like receptor (TLR) signaling pathways in hematopoietic stem and progenitor cells (HSPCs), which has been recently reviewed.¹ In particular, TLR-4 activation contributes to HSPC cell death in MDS.^{2,3} Additionally, over-activation of downstream components of the TLR-4 pathway, including Interleukin Receptor Associated Kinase-1 (IRAK1) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), play a prominent role in MDS pathogenesis, specifically in patients with deletion 5q (del(5q)) MDS. Specifically, del(5q) leads to *miR-145* and *miR-146* haploinsufficiency with consequent de-repression of *TRAF6* and *IRAK1* leading to the hallmark features of del(5q) MDS.³⁻⁵ Additionally, *IRAK1* overexpression occurs in non-del(5q) MDS and most importantly targeted inhibition was selectively cytotoxic to MDS HSPCs while sparing normal HSPC.⁶

Although innate immune activation via TLR signaling has been well described, the specific inflammatory intermediaries leading to activation of these pathways remained elusive. In this regard, the DAMP proteins S100A8 and S100A9 heterodimerize to form calprotectin and serve as endogenous ligands for TLR-4 (Fig. 1).⁷ Notably, S100A8/A9 profoundly expands CD33⁺/Lin⁻/HLA-DR⁻ MDSCs in the bone marrow of MDS patients. MDSC in turn secrete S100A8/9, leading to autocrine and paracrine stimulation and propagation of inflammatory signals.⁸ S100A9 engagement of its cognate Siglec-3 (sialic acid binding Ig-like lectin 3) receptor, CD33, on MDSCs ultimately leads to impaired hematopoiesis via production of immunosuppressive cytokines interleukin 10 (IL-10) and transforming growth factor- β (TGF- β) and mobilization

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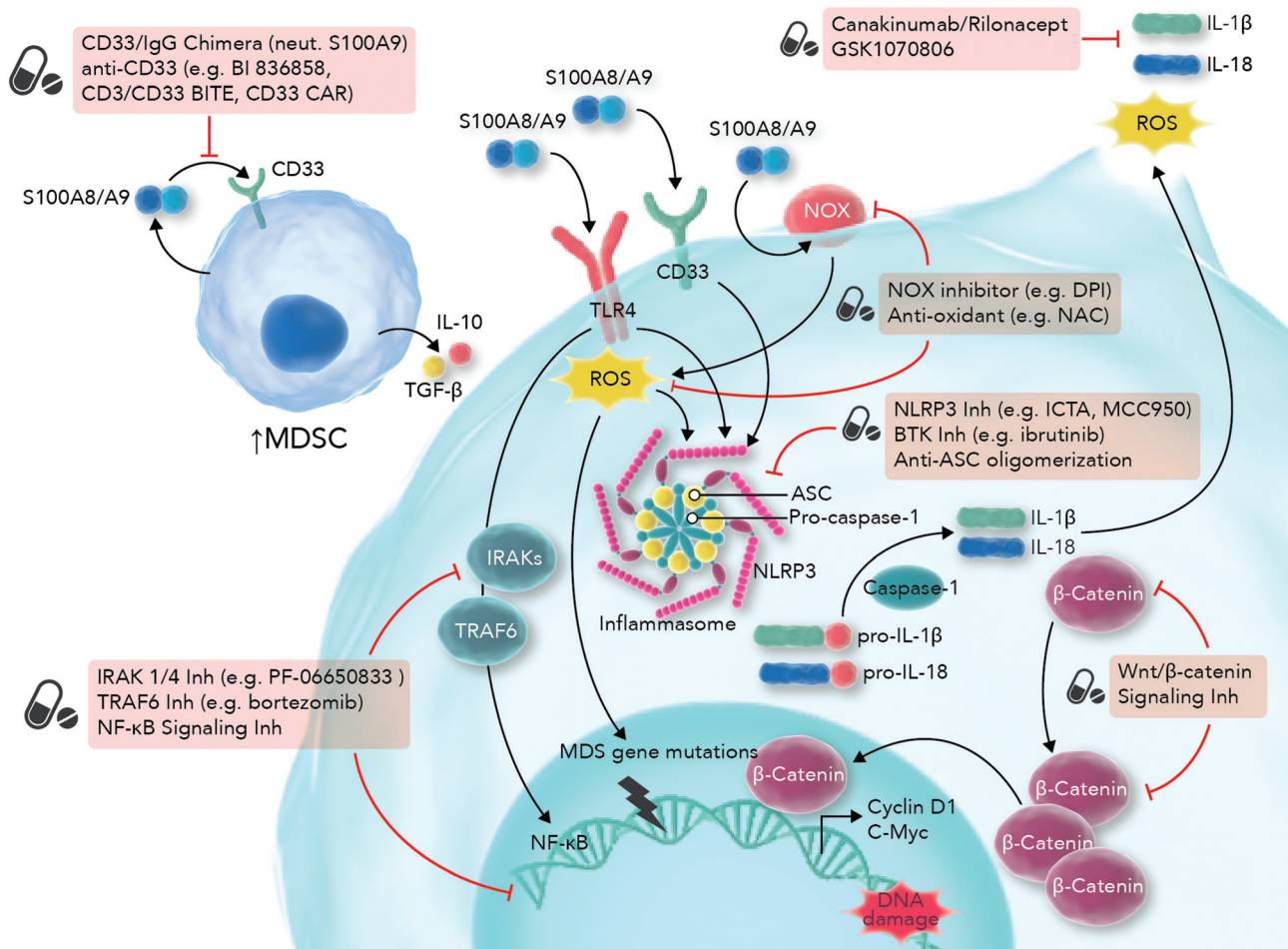


Figure 1. Targeting innate and inflammatory signaling for the treatment of MDS. ASC=associated speck-like protein containing a caspase-recruitment domain, DAMPs=damage-associated molecular patterns, IL=interleukin, IRAK=IL-1 receptor-associated kinases, Inh=inhibitor, neut=neutralize, NOX=dihyronicotinamide-adenine dinucleotide phosphate oxidase, TGF-b=transforming growth factor-b, TLR=Toll-like receptor, TRAF6=tumor necrosis factor receptor-associated factor 6. Adapted from Sallman et al. with permission¹¹.

of granzyme granules.⁹ Supporting MDSCs role as a potential disease initiating cellular effector, in a S100A9 transgenic mouse model, MDSC accumulation over time was accompanied by development of an age-dependent, impairment in hematopoiesis that phenocopied human MDS that was reversed by MDSC depletion or inhibition of the S100A9/CD33/TLR4 axis. Notably, MDSCs in primary bone marrow samples of MDS patients are genetically distinct and lack the classic somatic mutations found in the MDS clone. Additional supportive evidence that the bone marrow microenvironment and S100A8/A9-TLR activation can play a disease initiating role is provided through investigation of the role of mesenchymal niche cells. Utilizing a mouse model of the pre-leukemic disorder Schwachman-Diamond syndrome (SDS), *Sbds* gene deletion in osteoid progenitors led to activation of the p53-S100A8/9-TLR axis with consequent oxidative genotoxicity in HSPCs and MDS transformation.¹⁰

Aberrant innate immune activation is initiated not only through the bone marrow microenvironment, but also directly as a result of cell-intrinsic genetic alterations.¹¹ In a ribosomal protein small subunit 14 (*RPS14*) haploinsufficient mouse model that phenocopies the dyserythropoiesis in del(5q) MDS, p53-S100A8/9-TLR4 activation resulted in erythroid cell death while inhibition of this pathway restored erythropoiesis.¹² Additionally, this inflammatory circuit is activated by common somatic mutation events such as *Srsf2*^{P95H}, which induces S100A9 over expres-

sion.¹³ Both *SRSF2* and *SF3B1* mutations induce NFκB hyperactivation, albeit via unique aberrant splicing events.¹⁴ Other somatic mutations have been shown to enumerate pro-inflammatory cytokines as evidenced by *TET2* inactivating mutations inducing IL-1β production.¹⁵ Lastly, overexpression of the epigenetic regulator *KDM6B*, which is a common event in MDS, leads to transcriptional activation of S100A9 with *KDM6B* inhibition restoring effective hematopoiesis.¹⁶

Although the above investigations clearly indicate a profound role of innate immune activation in MDS pathogenesis, the precise cell death mechanism leading to macrocytosis, clonal proliferation, and ineffective hematopoiesis was poorly understood. A significant advancement in this regard is the identification that NOD-like receptor protein 3 (NLRP3) inflammasome activation, via somatic mutations or S100A8/A9, and consequent pyroptosis directs the MDS phenotype (Fig. 1).^{11,17} S100A9 and inflammasome components are markedly elevated in primary HSPCs and S100A9 in primary MDS bone marrow samples was sufficient to induce pyroptosis. NLRP3 inflammasome activation generates excess reactive oxygen species, Wnt/β-catenin-induced proliferation, cation flux-induced cell swelling with ultimately caspase-1 activation and inflammatory cytokine production (ie, IL-1β and IL-18). More importantly, neutralization of S100A9 and/or pharmacologic inhibition of inflammasome assembly restored effective hematopoiesis in vitro

as well as in the S100A9 transgenic mouse model. Highlighting the broad importance of inflammasome assembly in MDS, the formation of apoptosis-associated speck like protein containing a caspase-recruitment domain (ASC) specks has been recently identified as a robust diagnostic biomarker with a sensitivity and specificity of 84% and 87%, respectively.¹⁸ Specifically, peripheral blood ASC speck % was markedly elevated in MDS patients (lower risk > higher risk) and significantly higher in comparison to age-matched healthy controls or patients with other hematologic malignancies. Lastly, age-related clonal hematopoiesis, a precursor to overt MDS, has high cardiovascular morbidity/mortality which has been shown to be driven by NLRP3 inflammasome activation and IL-1 β secretion in inflammatory macrophages with suppression of atherosclerosis mediated by NLRP3 inhibition.^{19,20}

Future perspective

Delineation of the role of innate immune activation, the bone marrow microenvironment, and pyroptosis has significantly advanced our understanding of MDS disease pathogenesis. More importantly, these investigations provide strong rationale for novel therapeutic strategies to not only restore effective hematopoiesis but to also biologically-rational, disease modifying agents, thereby providing optimism for the treatment landscape of MDS patients (Fig. 1).²¹

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