HEMATOPOIESIS

Fueling clonal dominance through TRAFficking of NF-κB signaling

Activation of TLR–TRAF6 signaling by chronic inflammation in myelodysplastic syndromes increases the competitive advantage of HSPCs harboring MDS mutations through the upregulation of the ubiquitin-modifying enzyme A20 and a switch from canonical to non-canonical NF-κB signaling.

Koki Ueda, Rajni Kumari and Ulrich Steidl

In this issue of *Nature Immunology*, Muto et al. uncover novel molecular and cellular mechanisms of cooperativity between inflammation and innate immune signaling in the development of clonal dominance and the progression of myelodysplastic syndromes (MDSs)[1]. MDSs are diseases derived from altered hematopoietic stem and progenitor cells (HSPCs) that harbor various precancerous mutations (in genes such as *TET2, DNMT3A, EZH2, ASXL1, del5q, U2AF1, SF3B1, SRSF2* and *KDM6B*) and are associated with the activation of innate immune signaling[2–4]. Although a relationship between the microenvironment-associated systemic inflammation and the progression of MDS has been established[2], the molecular and cellular mechanisms of how MDS HSPCs selectively acquire a clonal advantage over normal HSPCs are still largely unclear. Muto et al. report that overexpression and activation of the adaptor protein TRAF6 in MDS HSPCs alters the response to inflammation in these cells by causing a switch to non-canonical signaling through upregulation of the zinc finger protein A20 to activate the transcription factor NF-κB, leading to a competitive advantage for MDS HSPCs as compared to normal HSPCs under chronic inflammatory conditions[1] (Fig. 1).

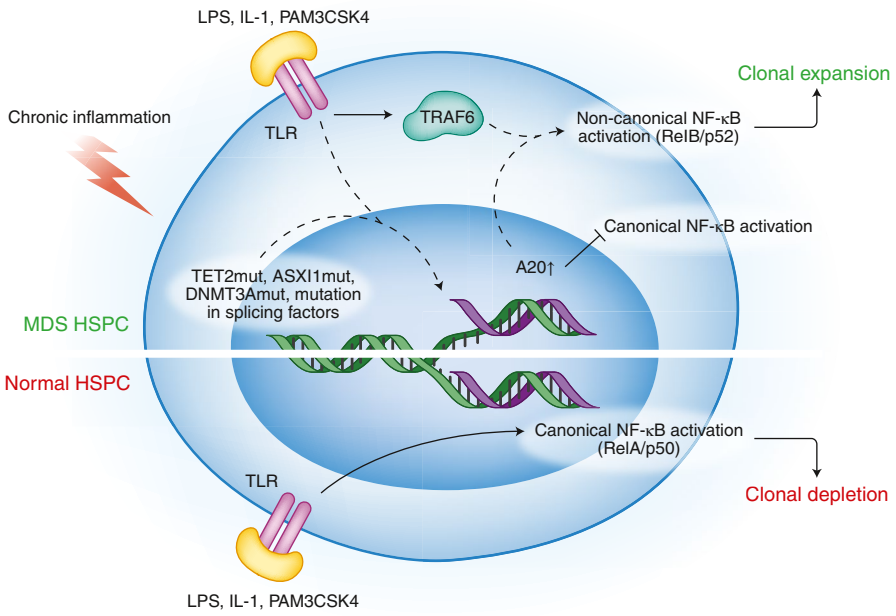

Fig. 1 | Overexpression of TRAF6 and systemic inflammation support clonal dominance in MDS. Upon induction of chronic inflammation, stimulated by low-dose-LPS (LD-LPS) injection in mice, overexpression of TRAF6 in MDS HSPCs preferentially activates non-canonical NF-κB signaling, which leads to the clonal expansion of MDS HSPCs. Induction of a chronic inflammatory response activates canonical NF-κB signaling in normal HSPCs, which negatively impacts their survival.

The expansion of MDS HSPCs and the development of clonal dominance have been reported previously in human MDS patient samples and several MDS murine models[4–8]. However, MDS HSPCs show insufficient growth in vitro and poor engraftment in xenograft models as compared to normal HSPCs, suggesting an intrinsic weakness[9,10]. Because systemic inflammation is one of the hallmarks of the MDS microenvironment, Muto et al. performed an elegant series of experiments that focused specifically on the differential response to inflammatory signals between MDS and normal HSPCs to interrogate the molecular mechanism of this functional discrepancy. Among various innate immune signaling mediators, TNF-receptor-associated TRAF6 plays a central role in regulating signaling through the Toll like receptors (TLRs) in MDS[2,8]. TRAF6, a member of both the TNF receptor superfamily and the Toll/interleukin (IL)-1 family, is overexpressed in most MDS patients[8]. Muto et al. found that overexpression of TRAF6 is associated not only with poor prognosis of MDS but also with the enrichment of inflammatory and immune-related gene expression signatures. To experimentally mimic the activation of immune response pathways in MDS HSPCs, the authors utilized Vav-TRAF6 transgenic mice, in which TRAF6 is specifically overexpressed in hematopoietic cells. Additionally, to mimic the chronic inflammatory milieu in MDS, these mice were stimulated with a low dose of lipopolysaccharide (LD-LPS), a TLR4 ligand that triggers a systemic inflammatory reaction. While overexpression of TRAF6 alone was insufficient to induce any inflammatory changes in vivo, treatment of Vav-TRAF6 HSPCs with LD-LPS in vitro elicited the expression of inflammatory-state-related genes. Consequently, overexpression of TRAF6 had a negative effect on the competitive repopulation capacity of Vav-TRAF6 bone marrow (BM) cells as compared to wild-type

cells in a primary transplantation assay. However, transplanted Vav-TRAF6 BM cells outcompeted wild-type BM cells in the myeloid and HSC fractions when recipient mice were also injected with LD-LPS. Importantly, the LPS-induced competitive advantage of Vav-TRAF6 BM cells in the primary transplantation persisted in serial transplantations, even without further induction of low-grade inflammation, while wild-type HSPCs exposed to LD-LPS lost their competitive advantage in further serial transplantations. Collectively, these findings indicate that HSPCs overexpressing TRAF6 are intrinsically more competitive and exhibit a serial clonal advantage under inflammatory conditions as compared to wild-type HSPCs. These observations suggest that MDS HSPCs, which have diminished repopulation efficiency under steady-state conditions, can outcompete normal HSPCs in MDS patients and murine models due to the inflammatory microenvironment in MDS[4–8].

Subsequently, Muto et al. investigated the molecular mechanisms underlying the distinct responses to chronic inflammatory signals and the varying competitiveness of wild-type and Vav-TRAF6 HSPCs. Among the pathways representing inflammatory signaling, the NF-κB pathway was significantly upregulated in both wild-type and Vav-TRAF6 HSPCs. A gene signature for non-canonical NF-κB signaling was differentially upregulated in Vav-TRAF6 HSPCs as compared to wild-type HSPCs, confirmed by the enhanced nuclear translocation of the transcription factor RelB in Vav-TRAF6 HSPCs. Intriguingly, expression of TRAF6 correlated with a non-canonical NF-κB gene signature in the HSPCs of patients with MDS, suggesting the relevance of the pathway in humans.

Because TRAF6 can activate both the canonical and non-canonical NF-κB pathways, Muto et al. further investigated the mechanism responsible for switching the NF-κB signaling pathway in Vav-TRAF6 HSPCs. To this end, they analyzed differentially expressed genes in three TLR–TRAF6-activated contexts, including HSPCs from *TRAF6*^{hi} MDS patients and *TRAF6*^{lo} patients, LPS-stimulated miR146-deficient human HSPCs and wild-type HSPCs (miR146 is a negative regulator of TRAF6 and TLR signaling) and LPS-stimulated Vav-TRAF6 HSPCs and wild-type HSPCs. Among the four genes (*CXCL1, CXCL2, CXCL3* and *TNFAIP3* (which encodes A20)) commonly upregulated in all of these TLR–TRAF6-activated contexts, the authors focused on the ubiquitin-modifying enzyme A20, which is known to suppress canonical signaling through NF-κB by various mechanisms and to activate non-canonical signaling by activating the NF-κB-inducing kinase NIK[11]. They also found that A20 was overexpressed in BM cells after stimulation with inflammatory mediators such as the TLR2 agonist PAM3CSK4 and IL-1, suggesting that a broad spectrum of inflammatory stimuli can contribute to the switch between canonical and non-canonical NF-κB signaling in MDS. The authors next tested whether similar effects were present in preleukemic *Tet2*$^{-/-}$ mice, because mutations in the methylcytosine dioxygenase TET2 in MDS are known to provoke inflammatory reactions[12]. A20 was indeed overexpressed in *Tet2*$^{-/-}$ HSPCs without LPS stimulation, and LPS-induced A20 upregulation in *Tet2*$^{-/-}$ BM cells was mediated by TRAF6.

One putative mechanism for how non-canonical NF-κB signaling induces a clonal advantage in MDS HSPCs could be that it reduces signaling through canonical NF-κB (RelA/p50), as the activation of canonical NF-κB is related to cellular apoptosis[13], and this is assumed to be the mechanism that impairs the function of normal HSPCs under inflammatory conditions. Signaling through non-canonical NF-κB (RelB/p52) has not been reported to activate apoptosis. Therefore, although both normal HSPCs and MDS HSPCs are negatively affected by chronic inflammation, MDS HSPCs could be more resilient and restorable due to their ability to switch to a non-canonical NF-κB response. While the authors utilized activation of the TLR–TRAF6 pathway and upregulation of A20 to induce the switch from canonical to non-canonical NF-κB as a representative pathway for the chronic inflammatory conditions in MDS, NF-κB switching may be a common phenomenon in MDS HSPCs. In addition to frequent mutations and/or overexpression of innate-immune-related genes that disrupt NF-κB signaling[14], other common mutations in MDS (in *DNMT3A, TET2* and *ASXL1*) disrupt TLR–TRAF6 signaling, through various mechanisms[2].

Overall, Muto et al. show that, due to a chronically inflamed microenvironment, preleukemic mutations and overexpression of TRAF6 in MDS upregulate TLR–TRAF6 signaling in HSPCs, and this activation leads to a switch from canonical to non-canonical NF-κB though upregulation of A20, a central regulator of NF-κB signaling. The selectivity of A20 in upregulating non-canonical NF-κB in MDS HSPCs supports the clonal expansion of these cells. These findings unveil a mechanism for how the intrinsically 'weak' and functionally impaired MDS HSPCs can acquire clonal advantage over normal HSPCs in an inflammatory context. This study also highlights that the phenomenon of clonal dominance in MDS is the result of both cell-extrinsic and cell-intrinsic pressures on HSPCs. The authors also present a molecular mechanism that could potentially be targeted therapeutically for selective inhibition of clonal dominance in MDS. A20-targeted therapy could be an attractive strategy for patients with MDS because it is not overexpressed in normal HSPCs. Because reversing NF-κB signaling from the non-canonical to the canonical pathway is supposed to sensitize MDS HSPCs to apoptosis, combinatorial therapies that include inhibitors of BCL2 (negative regulator of apoptosis) or MDMX/MDM2 (suppressors of p53) might also be worth considering in the future. Given the consistent activation of TLR–TRAF6 signaling during systemic inflammation in the aged bone marrow microenvironment, it will be interesting to examine whether the switch from canonical to non-canonical NF-κB signaling plays a role in other hematopoietic pathologies, including the ones arising from age-related clonal hematopoiesis. ❐

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Competing interests

The authors declare no competing interests.