Myelodysplastic syndrome

Activation of NF-κB driven inflammatory programs in mesenchymal elements attenuates hematopoiesis in low-risk myelodysplastic syndromes

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Highlights

- Activation of NF-κB signaling in mesenchymal cells is common in LR-MDS.
- Activation of NF-κB in mesenchymal cells leads to transcriptional overexpression of inflammatory factors including negative regulators of hematopoiesis.
- Activation of NF-κB attenuates HSPC numbers and function ex vivo.

Myelodysplastic syndromes (MDS) are clonal disorders characterized by ineffective hematopoiesis and the propensity for leukemic transformation. Cumulating evidence has challenged the traditional view that MDS is exclusively driven by hematopoietic cell intrinsic factors. Mesenchymal cells in the bone marrow (BM) microenvironment have emerged as key players in disease pathogenesis, as either initiating or contributing factors [1–4]. We have earlier demonstrated that the transcriptional landscape of highly purified mesenchymal elements from human low-risk MDS (LR-MDS) is distinct from normal mesenchymal cells and characterized by cellular stress and the upregulation of inflammatory molecules with known inhibitory effects on normal hematopoiesis [4]. Specifically, mesenchymal overexpression of the alarmins S100A8/9 was shown to drive genotoxic stress in hematopoietic stem/progenitor cells (HSPCs) and is related to leukemic evolution in a subset of LR-MDS patients [3]. An important question emerging from these findings is the nature of the upstream drivers of cellular stress and inflammatory programs in LR-MDS mesenchyme. Here, we show that activation of NF-κB in mesenchymal cells is common in LR-MDS, driving transcriptional activation of inflammatory programs and attenuating HSPC function.

We earlier reported on the elucidation of the transcriptome of highly purified mesenchymal cells isolated from LR-MDS patients (n = 45) by massive parallel RNA sequencing [3], suggesting inflammation in these mesenchymal elements. In order to identify candidate master regulatory pathways upstream of inflammatory programs in LR-MDS, we performed Gene Set Enrichment Analysis (GSEA) comparing the transcriptomes of these 45 patients to mesenchymal cells purified from healthy controls (n = 10) [4]. In total, 120 gene signatures were significantly enriched in the LR-MDS mesenchyme, while 8 signatures were enriched in normal mesenchymal cells. Among the signatures upregulated in LR-MDS patients was a remarkable abundance of signatures related to the activation of the nuclear factor-kappa B (NF-κB) family of transcription factors (Fig. 1a, b). To corroborate the notion of activation of this pathway and provide better insight into the heterogeneity within the population, we assessed the expression levels of NF-κB inhibitor NFKBIA (also known as IκB-α),
which forms an autoregulatory loop with activated NF-κB transcription factors and therefore directly reflects activation of NF-κB signaling [5, 6]. Overexpression of NFKBIA was found in the majority of patients, suggesting that mesenchymal NF-κB activation is a common feature in LR-MDS (Fig. 1c). To confirm the functional activation of NF-κB in mesenchymal elements in LR-MDS, we demonstrated increased phosphorylation of p65, a component of the
activated NF-κB complex, in intramedullary located CD271+ mesenchymal cells (Fig. 1d) as well as in bone-lining CD271+ stromal cells (Fig. 1e, f). Moreover, pathway analysis (GSEA) confirmed the transcriptional activation of NF-κB signaling in patients with increased NFκBIA expression in their mesenchymal niche cells (Figure S1A and S1B). NFκBIA expression was significantly correlated with the expression of inflammatory cytokines and negative regulators of hematopoiesis, which are bona fide NF-κB downstream targets such as IL6, IL8, and CCL3 (Figure S1C). No correlation was found between NFκBIA expression and expression of S100A8 or S100A9 (spearman correlation −0.11 and −0.22; P-adjusted 0.62 and 0.28, respectively). Patients with activated NFκB signaling (NFκBIA+) in mesenchymal niche cells had no significant difference in overall or progression-free survival in comparison to the NFκBIA− subset (Figure S2A and S2B) in this cohort of uniformly treated LR-MDS patients [4]. No significant correlations were found between mutational status and activation of NF-κB signaling in mesenchymal cells (Figure S2C).

While the genes encoding inflammatory factors and negative regulators of hematopoiesis [4] are bona fide downstream targets of NF-κB signaling in other experimental settings, we next wanted to provide experimental support for the view that NF-κB activation specifically in mesenchymal precursor cells results in upregulation of these targets. To this end, we designed a strategy of activating NF-κB signaling in mesenchymal progenitor cells by stably overexpressing the constitutively active form of IKK2 (FLAG-IKK2SE), a kinase upstream regulator of NF-κB, via a lentiviral vector (Fig. 2a, Figure S3A-C) [7] in OP9 cells. OP9 cells, like CD271+ cells [4], express osteo lineage commitment markers as well as HSPC regulatory factors and robustly support the expansion of human HSPCs [8]. NF-κB activation in OP9 cells resulted in overexpression of NFκBIA (Fig. 2c) and canonical NF-κB downstream negative regulators of hematopoiesis, including Il6, Cxcl2 (murine homolog of IL8), Ccl3, Inhba, Fth1, Lf, Ccl5, and Cxcl4 (Fig. 2b), recapitulating the findings in LR-MDS patients. Similar to the results in OP9 cells, activation of NF-κB in human mesenchymal cells (HS5 cell line and expanded bone-marrow-derived primary mesenchymal cells) (Figure S3D) also resulted in upregulation of NF-κB downstream targets including negative regulators of hematopoiesis such as IL6, IL8, CCL3, S100A9, INHBA, and CCL5 (Figure S3E). Together, these results extend the transcriptional landscape of inflammatory alterations in mesenchymal cells to activation of NF-κB in LR-MDS.

As earlier reported, in human MDS, the majority of CD34+ HSPCs is in direct contact with CD271+ mesenchymal cells [9]. To assess the effect of NF-κB activation in mesenchymal cells on the biology of normal HSPCs, we performed co-culture experiments with bone marrow CD34+ HSPCs and OP9 cells transduced with IKK2SE or empty vector (EV) (Fig. 2d). Co-culture for 7 days on IKK2SE-transduced OP9 cells resulted in significantly reduced numbers of immunophenotypically defined HSPCs in comparison to EV-transduced mesenchymal cells (Fig. 2d). In addition, reduced HSPC number was reflected in a reduced number of CFU-Cs (Fig.2e), indicating attenuation of proliferation and maintenance in non-proliferative, serum free, culture conditions. The decreased numbers of immunophenotypically defined HSPCs in comparison to EV-transduced mesenchymal cells (Fig. 2d, left panel). However, equalizing mesenchymal cell numbers by arresting their proliferation (using irradiation and maintenance in non-proliferative, serum free, culture conditions) (Figure S4B) recapitulated the significantly reduced numbers of immunophenotypically defined HSPCs on IKK2SE-transduced OP9 cells in comparison to EV-transduced mesenchymal cells (Fig. 2d, right panel). This included the fraction enriched for long-term hematopoietic stem cells (LT-HSCs) defined by the markers CD34+CD38−CD45RA−CD90+, multipotent progenitor (MPP; CD34+CD38−CD45RA−CD90−), multipotential progenitor (MLP; CD34+CD38−CD45RA−CD90−), as well as primitive (CD34+CD38+) and committed CD34+CD38+ progenitor cells (Fig. 2d), indicating that mesenchymal NF-κB signaling attenuates HSPC number and function, at least partially, independent of its effect on mesenchymal cell proliferation.

Collectively, in this brief communication, we demonstrate that mesenchymal NF-κB activation is a common...
finding in LR-MDS patients leading to transcriptional upregulation of inflammatory programs associated with negative regulation of hematopoiesis and attenuation of HSPC numbers and function.

Demonstration of mesenchymal activation of NF-κB provides human disease relevance to a number of murine studies implicating NF-κB activation in ancillary cells to the pathogenesis of hematopoietic disease. NF-κB activation in non-hematopoietic cells has been shown to induce ‘MDS-like’ myeloproliferative disease (MPD) in mice [10]. In another study, NF-κB activation in bone marrow mesenchymal cells and endothelial cells, as a result of elevated levels of the microRNA miR-155, generated a persistent pro-inflammatory state of the bone marrow niche leading to an MPD-like disease in a Notch/RBPJ loss-of-function mouse model [11]. Our experimental data demonstrate that NF-κB activation in the mesenchyme attenuates normal hematopoiesis, which is of key relevance to LR-MDS characterized by cytopenia. The implication of mesenchymal NF-κB activation in the pathogenesis of MDS may also point toward common mechanisms between the pathogenesis of MDS and oncogenesis in other systems.
NF-κB-mediated chronic tissue inflammation has been shown to drive cancer initiation and progression via secretion of cytokines and soluble factors in models of several other forms of cancer [12, 13], including skin, prostate, and colon cancer. In these models, activation of NF-κB signaling, specifically in fibroblasts, promoted malignant features in heterotypic (pre)cancerous cells, supporting the hypothesis that mesenchymal inflammation may facilitate tumorigenesis in the hematopoietic system as well.

The activation of NF-κB signaling in mesenchymal cells in most LR-MDS patients raises intriguing questions about the events driving this activation. This includes the question whether mesenchymal cell-intrinsic alterations or extrinsic events are driving NF-κB activation. While the answer to this question remains speculative in the absence of experimental evidence (and may vary between patients), it is conceivable that primary alterations in hematopoietic elements drive activation of NF-κB in the mesenchymal niche.

This notion is supported by recent findings where activation of the NF-κB pathway in CD34+ HSPCs is implicated in MDS pathogenesis [14] and CD271+ mesenchymal cells co-localize with CD34+ HSPCs in the bone marrow section of MDS patients [9]. It is therefore reasonable to hypothesize that activated NF-κB pathway in HSPCs signals to the adjacent mesenchymal elements, resulting in NF-κB activation in mesenchymal cells. As NF-κB activation is likely maintained through autocrine/paracrine feedback signaling networks, other cellular types that anatomically localize with the activated HSPCs and mesenchymal elements could be involved as well, suggesting that diverse cellular components may participate in this crosstalk. The combined findings suggest that in LR-MDS, activation of NF-κB occurs in both hematopoietic and mesenchymal cells, likely through autocrine and paracrine feedback signaling networks, leading to a NF-κB-mediated inflammatory milieu in the LR-MDS bone marrow and an overexpression of a repertoire of secreted negative hematopoietic regulators. S100A8/9, recently shown by us to induce NF-κB activation and genotoxic stress in HSPCs and to be associated with an increased likelihood of leukemic transformation, was not correlated with NFKBIA expression in patients, indicating that its regulation is more complex and may include upstream TP53 activation [3].

Taken together, the findings support the notion that mesenchymal factors, in addition to hematopoietic cell autonomous characteristics, may be therapeutically targeted in LR-MDS and warrant ongoing experiments defining the contribution of NF-κB activation and inflammation to ineffective hematopoiesis and leukemic evolution in MDS.

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Author contributions ZP, SC, and MHGPR designed the studies; ZP, SC, SJFH, KK, JF, CVD, EMJB, AMM, and DJL-K performed the experiments and acquired the data; SC, RMH, EMJB, and MAS provided technical guidance and bioinformatical analysis; JNS provided helpful insights in immunohistochemical data analysis; EMPC and Avdl provided the patient material; ZP, SC, and MHGPR wrote the manuscript. All authors were involved in data interpretation and manuscript reviewing; MHGPR supervised the study.

Compliance with ethical standards
Conflict of interest The authors declare that they have no conflict of interest.

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