IN THE SPOTLIGHT

**Inhibition of HIF1α Signaling: A Grand Slam for MDS Therapy?**

Jiahao Chen and Ulrich Steidl

**Summary:** The recent focus on genomics in myelodysplastic syndromes (MDS) has led to important insights and revealed a daunting genetic heterogeneity, which is presentizing great challenges for clinical treatment and precision oncology approaches in MDS. Hayashi and colleagues show that multiple mutations frequently found in MDS activate HIF1α signaling, which they also found to be sufficient to induce overt MDS in mice. Furthermore, both genetic and pharmacologic inhibition of HIF1α suppressed MDS development with only mild effects on normal hematopoiesis, implicating HIF1α signaling as a promising therapeutic target to tackle the heterogeneity of MDS. Cancer Discov; 8(11): 1355–7. © 2018 AACR.

See related article by Hayashi et al., p. 1438 (5).

Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic disorders characterized by inefficient hematopoiesis that manifests as bone marrow (BM) dysplasia and peripheral blood cytopenias, and increased risk of leukemia transformation (1). The median survival of patients with MDS is 4.5 years, with a significantly worse survival of <1 year within higher-risk subsets (1). Although several therapeutic agents, such as immunomodulatory drugs (e.g., lenalidomide) and hypomethylating agents (e.g., azacytidine and decitabine), have been approved for the treatment of patients with MDS, refractory disease or relapse occurs in most patients, who then have a median overall survival of less than 6 month (2).

Recent sequencing studies of MDS BM have revealed the genetic heterogeneity of the disease (3), with mutated genes involving splicing factors (mutated in ~60% of MDS; e.g., SF3B1, SRSF2, U2AF1, and ZRSR2), epigenetic regulators (~50%; e.g., TET2, ASXL1, DNMT3A, EZH2, and IDH1/2), transcription factors (~25%; e.g., RUNX1, TP53, and ETV6), kinase signaling (~15%; e.g., JAK2, NRAS, Kras, and CBL), and others. Certain mutations tend to (but by no means always) co-occur in the same patient (e.g., SRSF2, ASXL1, STAG2, and RUNX1 mutations—or are typically mutually exclusive; for example, SF3B1 mutations only rarely co-occur with other frequently mutated genes except DNMT3A and JAK2). These and similar studies have indicated the daunting genetic heterogeneity across different patients with MDS, as well as the coexistence of numerous smaller subclones in individual patients, which has been a major challenge in the clinical management of patients with MDS (4).

In this issue, Hayashi and colleagues found that target genes activated by HIF1α signaling were highly expressed in a published cohort of 183 patients with MDS compared with healthy controls (5). IHC confirmed an increased frequency of HIF1α-expressing cells in MDS samples at different stages (low-, intermediate-, and high-risk) compared with healthy controls. Interestingly, they also observed increased HIF1α activation in patients harboring several quite different mutations (e.g., DNMT3A, SF3B1, and/or U2AF1), which led the authors to examine whether different MDS-relevant mutations play a role in the activation of HIF1α signaling. Indeed, increased stabilization of HIF1α was consistently observed in sorted c-Kit+ cells from knockout models of multiple genes frequently mutated in MDS, including Dnmt3aΔΔ/ΔΔ mice, Tet2ΔΔ/ΔΔ mice, Runx1ΔΔ/ΔΔ mice, and Asxl1ΔΔ/ΔΔ mice, as well as heterozygous knock-in mice of MLL partial tandem duplication (PTD; MllΔΔ/ΔΔ). These results suggested that HIF1α activation may be a common downstream event mediating the molecular effects of multiple frequent mutations in MDS. Hayashi and colleagues then set out to test whether HIF1α activation itself is pathogenic, using a hematopoietic cell–specific Vav1-Cre transgenic model carrying a constitutively active human HIF1α triple-point mutant (HIF1A-TPM; P402A, P564A, and N803A) and its dimerization partner ARNT (also known as HIF1β). Remarkably, the HIF1A-TPM led to rapid development of MDS phenotypes in nearly all mice, displaying thrombocytopenia, leukocytopenia, anemia, as well as splenomegaly and multilineage dysplasia in the BM. The HIF1A-TPM–driven MDS phenotype was transplantable with BM cells, which also showed growth advantage in competitive BM transplantation assays compared with wild-type (WT) cells.

To interrogate the molecular mechanism of HIF1α activation following MDS-associated mutations, Hayashi and colleagues first performed H3K4me3 chromatin immunoprecipitation sequencing analysis of lineage Sca1–c-Kit+ cells (LSK) isolated from MllΔΔ/ΔΔ mice compared with WT mice. Interestingly, the increased H3K4me3-binding peaks in MllΔΔ/ΔΔ LSKs were highly enriched for Hif1α/Arnt heterodimer binding motifs, confirming at the molecular level that MLL-PTD mutations indeed affected HIF1α-mediated regulation. Afterward, they...
examined whether the HIF1α stabilization observed in MllΔ/ΔWT cells was mediated by diminished activities of proteasomal degradation mediated by von Hippel–Lindau tumor suppressor (pVHL) or murine double minute-2 (MDM2), which are two major ligases regulating the ubiquitination of HIF1α (6, 7). However, Hayashi and colleagues did not observe downregulation of Vhl or Mdm2. Therefore, the authors next interrogated the potential involvement of prolyl hydroxylase (PHD), the central regulator that hydroxylates HIF1α before recognition by pVHL-mediated ubiquitination (6). The catalytic activity of PHD is dependent on α-ketoglutarate (α-KG), an intermediate metabolite of the tricarboxylic acid (TCA) cycle (6). And accumulation of subsequent products following α-KG in the TCA cycle, e.g., succinate, fumarate, and malate, can induce pseudohypoxia and increase HIF1α stabilization by inhibiting the activity of PHD (6). Therefore, Hayashi and colleagues measured mitochondrial respiration, which has been associated with accumulation of TCA cycle intermediates, using c-Kit+ cells isolated from MllΔ/ΔWT and WT mice. Interestingly, both mitochondrial respiration and activities of mitochondrial complexes I/II/III were significantly decreased in MllΔ/ΔWT mice compared with WT mice. In addition, multiple genes encoding for components of complex II, including Sdha/b/d, were significantly downregulated in MllΔ/ΔWT cells. Notably, increased amounts of succinate, fumarate, and malate as well as pyruvate were detected in the plasma of MllΔ/ΔWT mice compared with WT mice, and cells cultured with exogenous succinate, fumarate, and malate also showed increased stabilization of HIF1α even under normoxic conditions. Together, these results demonstrated that MLL-PTD mutations induce a pseudohypoxia condition, leading to inhibition of PHD activity and hence decreased pVHL-mediated HIF1α degradation.

Importantly, these findings also provide a direct mechanistic link of HIF1α-inducing pseudohypoxia with the genome-wide hypermethylation phenotype that is a hallmark of MDS.

Subsequently, to interrogate whether HIF1α activation is required for MDS development following the MDS-associated mutations, Hayashi and colleagues first crossed Hif1αΔ/Δ mice with MllΔ/ΔWT mice. The MllΔ/ΔWT mice did not develop overt hematologic malignancy but showed MDS-associated cellular phenotypes, including increased self-renewal and a differentiation block of HSCs, as well as expansion of the myeloid progenitors. Interestingly, although Hif1α+/− mice did not show pronounced changes in steady-state hematopoiesis, knockout of Hif1α restored the increased in vitro serial reconstituting capacity and in vivo serial hematopoietic reconstitution of MllΔ/ΔWT cells. To investigate the role of HIF1α activation in the pathogenesis of overt MDS, Hayashi and colleagues first demonstrated that the cooperation of MllΔ/ΔWT with Runx1 mutations, Runx1Δ/Δ, induced full-blown MDS. The authors thereafter established a new genetic model of overt MDS by crossing MllΔ/ΔWT with Runx1 knockout (Runx1Δ/Δ) mice, and these compound mutant mice rapidly developed transplantable MDS and died within 3 months. The overexpression of HIF1α protein in MllΔ/ΔWT/Runx1Δ/Δ and MllΔ/ΔWT/Runx1Δ/Δ cells was around 1.5-fold higher than in MllΔ/ΔWT cells, which could be explained by the additive effect of downregulation of MDM2-mediated degradation caused by Runx1 mutation or knockdown. Interestingly, the HIF1A-TPM MDS model led to the same level of HIF1α accumulation as MllΔ/ΔWT/Runx1Δ/Δ and MllΔ/ΔWT/Runx1Δ/Δ, suggesting that the exact dose of HIF1α activation may be critical in the development of full-blown MDS. Remarkably, additional deletion of Hif1α rescued the multilineage dysplasia in the BM and significantly prolonged the survival of MllΔ/ΔWT/Runx1Δ/Δ mice. Together, these results demonstrated an essential role of HIF1α activation in the pathogenesis of MDS, suggesting HIF1α as promising novel therapeutic target in MDS.

To this end, Hayashi and colleagues also examined the effect of a pharmacologic inhibitor of HIF1α, echinomycin, for the treatment of murine MDS (MllΔ/ΔWT/Runx1Δ/Δ and MllΔ/ΔWT/Runx1Δ/Δ), as well as human MDS-derived cells (MDS-L). Although treatment had only mild effects on WT BM cells, echinomycin significantly diminished the colony-forming capacity of MllΔ/ΔWT/Runx1Δ/Δ MDS cells. Furthermore, echinomycin treatment significantly extended the survival of mice transplanted with MllΔ/ΔWT/Runx1Δ/Δ MDS cells. Notably, echinomycin was also effective in a xenotransplantation model of human MDS cells.

In summary, in this study, Hayashi and colleagues demonstrate that HIF1α signaling is highly activated in patients with MDS, and HIF1α activation is sufficient and required to induce overt MDS in mice. Importantly, genetic and pharmacologic inhibition of HIF1α suppressed the development and growth of MDS with only mild effects on normal hematopoiesis (Fig. 1). Together with the finding that HIF1α signaling is activated downstream of multiple frequent MDS-relevant mutations, this study implicates HIF1α as a promising novel therapeutic target in MDS and suggests its broad efficacy for the treatment of MDS with diverse genetic lesions (Fig. 1). It will be interesting to assess in future studies whether HIF1α inhibition also suppresses the growth of primary cells from newly diagnosed MDS, as well as relapsed MDS. Moreover, as the authors also found HIF1α activation in patients with splicing factor mutations, it will be interesting to study whether HIF1α is necessary for the pathogenesis of these subsets of MDS. Because of the uncovered mechanistic link between HIF1α-inducing pseudohypoxia and genome-wide hypermethylation, it is also tempting to speculate that the efficacy of hypomethylating agents in MDS is, at least in part, due to this mechanism. Thus, HIF1α-targeted (combination) therapy may be interesting to test in patients with MDS resistant to hypomethylating agents or IDH-mutant inhibitors. Lastly, previous studies have demonstrated the crucial role of MDS stem cells in the development and progression of MDS (8–10). The fact that Hayashi and colleagues observed increased HIF1α prior to the development of full-blown MDS, as well as in multiple models of clonal hematopoiesis-associated mutations, suggests HIF1α activation is a relatively early event during the initiation of MDS. Therefore, it may be interesting to interrogate the role of HIF1α specifically in clonal hematopoiesis and preleukemic and MDS stem cells in the future, to assess its potential utility as an MDS stem cell targeted therapy.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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Figure 1. Essential role of HIF1α activation in MDS pathogenesis and its therapeutic implications. Mouse models of alleles frequently mutated in individuals with clonal hematopoiesis or patients with MDS display activation of HIF1α signaling, but do not develop full-blown MDS. However, MllPTD/WT/Runx1Δ/Δ compound mice rapidly develop overt, transplantable MDS induced by a higher level of HIF1α activation, which is due to combined effects of both pseudohypoxia-mediated suppression of PHD hydroxylation and subsequent pVHL recognition, as well as downregulation of MDM2-mediated degradation. Remarkably, knockout of Hif1a (Hif1aΔ/Δ) or pharmacologic inhibition of HIF1α suppresses the development of MDS in MllPTD/WT/Runx1Δ/Δ mice. In addition, constitutive activation of HIF1α in mice (HIF1A-TPM), to an extent comparable to MllPTD/WT/Runx1Δ/Δ mice, also leads to rapid development of MDS, demonstrating that HIF1α activation is necessary and sufficient to induce MDS. The functional role of HIF1α in ASXL1/DNMT3A/TET2-associated MDS remains to be determined.

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