

IN THE SPOTLIGHT

Inhibition of HIF1 α Signaling: A Grand Slam for MDS Therapy?



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Summary: The recent focus on genomics in myelodysplastic syndromes (MDS) has led to important insights and revealed a daunting genetic heterogeneity, which is presenting great challenges for clinical treatment and precision oncology approaches in MDS. Hayashi and colleagues show that multiple mutations frequently found in MDS activate HIF1 α signaling, which they also found to be sufficient to induce overt MDS in mice. Furthermore, both genetic and pharmacologic inhibition of HIF1 α suppressed MDS development with only mild effects on normal hematopoiesis, implicating HIF1 α 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 leukemic 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., azacitidine and decitabine), have been approved for the treatment of patients with MDS, refractory disease or relapse occur 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).

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In this issue, Hayashi and colleagues found that target genes activated by HIF1 α signaling were highly expressed in a published cohort of 183 patients with MDS compared with healthy controls (5). IHC confirmed an increased frequency of HIF1 α -expressing cells in MDS samples at different stages (low-, intermediate-, and high-risk) compared with healthy controls. Interestingly, they also observed increased HIF1 α 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 HIF1 α signaling. Indeed, increased stabilization of HIF1 α was consistently observed in sorted c-Kit⁺ cells from knockout models of multiple genes frequently mutated in MDS, including *Dnmt3a* ^{Δ/Δ} mice, *Tet2* ^{Δ/Δ} mice, *Runx1* ^{Δ/Δ} mice, and *Asxl1* ^{Δ/Δ} mice, as well as heterozygous knock-in mice of *MLL* partial tandem duplication (PTD; *MLL*^{PTD/WT}). These results suggested that HIF1 α activation may be a common crucial downstream event mediating the molecular effects of multiple frequent mutations in MDS. Hayashi and colleagues then set out to test whether HIF1 α activation itself is pathogenic, using a hematopoietic cell-specific *Vav1*-Cre transgenic model carrying a constitutively active human HIF1 α triple-point mutant (*HIF1A*-TPM; P402A, P564A, and N803A) and its dimerization partner ARNT (also known as HIF1 β). 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 HIF1 α activation following MDS-associated mutations, Hayashi and colleagues first performed H3K4me3 chromatin immunoprecipitation sequencing analysis of lineage⁻Sca1⁺c-Kit⁺ cells (LSK) isolated from *MLL*^{PTD/WT} mice compared with WT mice. Interestingly, the increased H3K4me3-binding peaks in *MLL*^{PTD/WT} LSKs were highly enriched for *Hif1a/Arnt* heterodimer binding motifs, confirming at the molecular level that MLL-PTD mutations indeed affected HIF1 α -mediated regulation. Afterward, they

examined whether the HIF1 α stabilization observed in *Mll*^{PTD/WT} 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 HIF1 α (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 HIF1 α 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 HIF1 α 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 *Mll*^{PTD/WT} and WT mice. Interestingly, both mitochondrial respiration and activities of mitochondrial complexes I/II/III were significantly decreased in *Mll*^{PTD/WT} mice compared with WT mice. In addition, multiple genes encoding for components of complex II, including *Sdhb/b/d*, were significantly downregulated in *Mll*^{PTD/WT} cells. Notably, increased amounts of succinate, fumarate, and malate as well as pyruvate were detected in the plasma of *Mll*^{PTD/WT} mice compared with WT mice, and cells cultured with exogenous succinate, fumarate, and malate also showed increased stabilization of HIF1 α 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 HIF1 α degradation. Importantly, these findings also provide a direct mechanistic link of HIF1 α -inducing pseudohypoxia with the genome-wide hypermethylation phenotype that is a hallmark of MDS.

Subsequently, to interrogate whether HIF1 α activation is required for MDS development following the MDS-associated mutations, Hayashi and colleagues first crossed *Hif1a*^{fllox/fllox} mice with *Mll*^{PTD/WT} mice. The *Mll*^{PTD/WT} 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 *Hif1a* ^{Δ/Δ} mice did not show pronounced changes in steady-state hematopoiesis, knockout of *Hif1a* restored the increased *in vitro* serial replating capacity and *in vivo* serial hematopoietic reconstitution of *Mll*^{PTD/WT} cells. To investigate the role of HIF1 α activation in the pathogenesis of overt MDS, Hayashi and colleagues first demonstrated that the cooperation of *Mll*^{PTD/WT} with *RUNX1* mutations, *RUNX1-S291fs:X300* (*RX1-S291fs*), induced full-blown MDS. The authors thereafter established a new genetic model of overt MDS by crossing *Mll*^{PTD/WT} with *Runx1* knockout (*Runx1* ^{Δ/Δ}) mice, and these compound mutant mice rapidly developed transplantable MDS and died within 3 months. The overexpression of HIF1 α protein in *Mll*^{PTD/WT}/*RX1-S291fs* and *Mll*^{PTD/WT}/*Runx1* ^{Δ/Δ} cells was around 1.5-fold higher than in *Mll*^{PTD/WT} 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 HIF1 α accumulation as *Mll*^{PTD/WT}/*RX1-S291fs* and *Mll*^{PTD/WT}/*Runx1* ^{Δ/Δ} , suggesting that the exact dose of HIF1 α activation may be critical in the development of full-blown MDS. Remarkably, additional deletion of *Hif1a* rescued the multilineage dysplasia in the BM and significantly prolonged the survival of *Mll*^{PTD/WT}/*Runx1* ^{Δ/Δ} mice. Together, these results demonstrated an essential role of HIF1 α activation in the pathogenesis of MDS, suggesting HIF1 α as promising novel therapeutic target in MDS. To this end, Hayashi and colleagues also examined the effect of a pharmacologic inhibitor of HIF1 α , echinomycin, for the treatment of murine MDS (*Mll*^{PTD/WT}/*Runx1* ^{Δ/Δ} and *Mll*^{PTD/WT}/*RX1-S291fs*), 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 *Mll*^{PTD/WT}/*Runx1* ^{Δ/Δ} MDS cells. Furthermore, echinomycin treatment significantly extended the survival of mice transplanted with *Mll*^{PTD/WT}/*RX1-S291fs* 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 HIF1 α signaling is highly activated in patients with MDS, and HIF1 α activation is sufficient and required to induce overt MDS in mice. Importantly, genetic and pharmacologic inhibition of HIF1 α suppressed the development and growth of MDS with only mild effects on normal hematopoiesis (Fig. 1). Together with the finding that HIF1 α signaling is activated downstream of multiple frequent MDS-relevant mutations, this study implicates HIF1 α 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 HIF1 α inhibition also suppresses the growth of primary cells from newly diagnosed MDS, as well as relapsed MDS. Moreover, as the authors also found HIF1 α activation in patients with splicing factor mutations, it will be interesting to study whether HIF1 α is necessary for the pathogenesis of these subsets of MDS. Because of the uncovered mechanistic link between HIF1 α -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, HIF1 α -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 graded increase of HIF1 α prior to the development of full-blown MDS, as well as in multiple models of clonal hematopoiesis-associated mutations, suggests HIF1 α activation is a relatively early event during the initiation of MDS. Therefore, it may be interesting to interrogate the role of HIF1 α 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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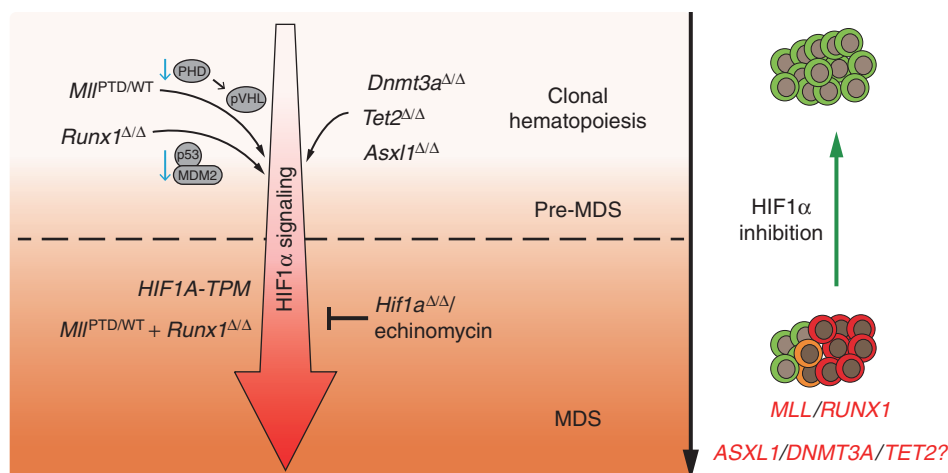


Figure 1. Essential role of HIF1 α activation in MDS pathogenesis and its therapeutic implications. Mouse models of alleles frequently mutated in individuals with clonal hematopoiesis or patients with MDS display activation of HIF1 α signaling, but do not develop full-blown MDS. However, *Mll*^{P^{TD}/WT}/*Runx1* ^{Δ/Δ} compound mice rapidly develop overt, transplantable MDS induced by a higher level of HIF1 α 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* (*Hif1a* ^{Δ/Δ}) or pharmacologic inhibition of HIF1 α suppresses the development of MDS in *Mll*^{P^{TD}/WT}/*Runx1* ^{Δ/Δ} mice. In addition, constitutive activation of HIF1 α in mice (*HIF1A*-TPM), to an extent comparable to *Mll*^{P^{TD}/WT}/*Runx1* ^{Δ/Δ} mice, also leads to rapid development of MDS, demonstrating that HIF1 α activation is necessary and sufficient to induce MDS. The functional role of HIF1 α in *ASXL1/DNMT3A/TET2*-associated MDS remains to be determined.

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