

Hypoxia-HIF-1 α -C/EBP α /Runx1 signaling in leukemic cell differentiation

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Abstract

Acute myeloid leukemia (AML), a class of prevalent hematopoietic malignancies, is caused by the acquisition of gene mutations that confer deregulated proliferation, impaired differentiation and a survival advantage of hematopoietic progenitors. More recently, we reported that cobalt chloride (CoCl₂)/iron chelator desferrioxamine (DFO)-mimicked hypoxia or moderate hypoxia (2% and 3% O₂) can directly trigger differentiation of many subtypes of AML cells. Also, intermittent hypoxia significantly prolongs the survival of the transplanted leukemic mice with differentiation induction of leukemic cells. Additionally, these hypoxia-simulating agents selectively stimulate differentiation in acute promyelocytic leukemic cells induced by arsenic trioxide, an effective second-line drug for this unique type of leukemia. Based on this interesting evidence *in vitro* and *in vivo*, the ongoing investigations showed the role of hypoxia-inducible factor-1 α (HIF-1 α) protein through its non-transcriptional activity in myeloid cell differentiation, as evidenced by chemical interference, the conditional HIF-1 α induction, the specific short hairpin RNAs (shRNAs) against HIF-1 α and HIF-1 β , an essential partner for transcription activity of HIF-1. Furthermore, HIF-1 α and two hematopoietic transcription factors CCAAT/enhancer binding protein alpha (C/EBP α) and Runx1/AML1 interact directly with each other. Such interactions increase the transcriptional activities of C/EBP α and Runx1/AML1, while C/EBP α competes with HIF-1 β for direct binding to HIF-1 α protein, and significantly inhibits the DNA-binding ability of HIF-1. As a protein is rapidly responsive to *all-trans* retinoic acid (ATRA), a classical clinical differentiation-inducing drug for AML, HIF-1 α also plays a role in ATRA-induced differentiation of leukemic cells.

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1. Introduction

Hematopoiesis is a delicately regulated process, during which hematopoietic stem cells (HSC) in the bone marrow give rise to committed progenitor cells, which in turn differentiate into various functional mature blood cells. Acute myeloid leukemia (AML), a class of prevalent hematopoietic malignancies, is characterized by the block of normal differentiation pathway at a stage where the cells continue to proliferate and do not move on to terminal differentiation, mainly due to specific gene rearrangements and

mutations targeting a variety of transcription factors and signaling molecules. For example, cells of acute promyelocytic leukemia (APL), a unique subtype of AML, are blocked at the stage of the promyelocytes due to the expression of chromosome translocation *t*(15;17), yielding the specific fusion protein PML-RAR α (for promyelocytic leukemia-retinoic acid receptor α), which interacts with transcriptional co-repressors such as the nuclear co-repressor (N-CoR)-histone deacetylase complex and exerts dominant-negative effects on functions of the wild type PML and RAR α proteins [1–3].

Based on the tacit assumption that AML cells exhibit reversible defects in their differentiation process, a potentially less cytotoxic therapeutic strategy for cancer known as ‘differentiation therapy’ is being developed. It employs drugs to induce cancer cells to undergo terminal differentiation, thus preventing their further proliferation. As early as

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the 1960–1970s, control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukemia were reported and differentiation of myeloid leukemia cells was put forward as new possibilities for therapy. Until 1980s, this differentiation therapy comes true from hypothesis for the first time, concomitant with the successful differentiation induction of *all-trans* retinoic acid (ATRA) in APL patients by Chinese doctors [4,5]. From then on, ATRA has always been the first-line drug for the APL patient in clinic and the mechanisms by which it induces differentiation with growth arrest as well as apoptosis have been provoking extensive studies in the past decades [6–8]. Despite earlier optimism, the concept of “differentiation therapy” has not yet been usefully extended to other subtypes of leukemia. Thus, it becomes even more urgent to explore practicable differentiation induction therapy in other leukemia subtypes besides APL.

Along with the world-wide use of ATRA in clinic, at the same time, some problems also emerge such as drug resistance in APL patients. To this end, arsenic trioxide (As_2O_3 , ATO, commercial name TrisenoxTM) is employed by Chinese doctors to treat relapsed/refractory APL patients [9], and the mechanisms through which it works have been gaining a tremendous amount of insight. A series of studies showed that clinical concentration of As_2O_3 (1–2 $\mu\text{mol/L}$) triggers apoptosis not only in APL with the down-regulation of bcl-2 gene expression and the modulation of PML-RAR α protein [9,10], but also in an impressive array of other cancer cells, in which various kinds of mechanisms have been documented [11–13]. However, the clinical effectiveness of As_2O_3 appears to be restricted to APL, which suggests that some other effects besides the apoptosis induction contribute to the efficacy of As_2O_3 in clinic. Further investigation indicated that after 2–3 weeks of continuous *in vivo* As_2O_3 treatment, myelocyte-like cells and degenerative cells in bone marrow and peripheral blood increase when leukemic promyelocytes decrease, which is probably as a result of partial *in vivo* differentiation [14,15]. This incomplete differentiation effect of As_2O_3 was confirmed by other clinical trials and in transplanted APL mice. On the other hand, *in vitro*, As_2O_3 has dose-dependent dual effects on APL cell lines: inducing apoptosis preferentially at relatively high concentrations (0.5–2 $\mu\text{mol/L}$) and inducing partial differentiation at low concentrations (0.1–0.5 $\mu\text{mol/L}$) [14,16], the latter, however, is not so obvious as that of As_2O_3 *in vivo*. Based on these observations, it is reasonable to assume that the partial differentiation induction of As_2O_3 also plays a role in its impressive clinical efficacy, and some factors in the bone marrow (BM) microenvironments could modulate the *in vivo* activity of As_2O_3 .

When we set to figure out the difference existing *in vivo* and *in vitro*, oxygen concentration comes into our consideration. As it is well known that *in vitro* leukemic cells are cultured in ambient O_2 concentration around 21%, while *in vivo*, tissues are exposed physiologically to lower O_2 tension, ranging from 16% in pulmonary alveoli and artery blood to

less than 5% in distal tissues. The O_2 tension of bone marrow in leukemic patients might be much lower due to the rapid and terrible proliferation of the leukemic cells [17]. Furthermore, increasing vascular endothelial growth factor (VEGF) levels, which is associated with angiogenesis, growth, dissemination, metastasis and poor outcome in solid tumors, is reported to be related to shorter survival as the independent predictor of the outcome in diagnosed AML [18,19]. According to these, we begin to broaden our view about the regulation of oxygen metabolism in the organism and to investigate whether oxygen concentration influences As_2O_3 -induced leukemic cell differentiation.

Cellular and systemic O_2 homeostasis, consisting of O_2 delivery and consumption, is essential for the survival of higher eukaryotes. As a consequence, delicate mechanisms are utilized to regulate O_2 delivery, among which, hypoxia-inducible factor-1 (HIF-1) plays an essential role [20]. HIF-1 is a heterodimeric transcriptional factor composed of the constitutively expressed HIF-1 β (also named aryl-hydrocarbon receptor nuclear transporter, ARNT) subunit and the highly regulated HIF-1 α subunit [21]. Under normoxia, the HIF-1 α subunit is hydroxylated by oxygen-activated HIF prolyl hydroxylases (PHDs) in the oxygen-dependent degradation domain (ODD) and rapidly degraded by the von Hippel-Lindau (VHL) tumor suppressor protein-mediated ubiquitin–proteasome pathway. On the contrary, under hypoxic or hypoxic-mimic condition, the HIF-1 α subunit accumulates due to the significantly reduced enzymatic activities of PHDs. The stabilized HIF-1 α is translocated to the nucleus, heterodimerized with HIF-1 β and activates an impressive array of target genes through binding to the hypoxia-responsive element (HRE) in the cis-acting sequences so as to overcome hypoxic stress.

Since first identified as a nuclear factor induced under hypoxia, HIF-1 has been attracting a tremendous amount of insight because of its involvement in fundamental biological processes, including but not limited to tumor metabolism, angiogenesis, metastasis, inflammation, and its potential role as a therapeutic target [22]. Although the roles of hypoxia in solid tumors have been widely studied, few studies are reported regarding the possible effects of hypoxia on leukemic cells. At the same time, the *in vitro* and *in vivo* observations mentioned above drive us to extend research of hypoxia to leukemia, which, surprisingly, uncover the two-edged sword effect of hypoxia/HIF-1 α . Herein, this review will focus on the recent research of the differentiation induction effects of hypoxia/HIF-1 α and the related pathways in leukemic cells.

2. Hypoxia induces AML cell differentiation: *in vitro* and *in vivo* evidence

Hypoxia-mediated modulation of hematopoietic progenitor behavior by oxygen tension has been reported [23,24]. Unexpectedly, on the way to unravel the possible effect of

As₂O₃ on APL cells under hypoxia, we found that mild hypoxia (2–3% O₂) and non-toxic concentrations of hypoxia-mimetic agents such as cobalt chloride (CoCl₂, 12.5–50 μM) and desferrioxamine (DFO, 5–20 μM) trigger differentiation in APL cell lines NB4, as assessed by morphological criteria and differentiation-associated antigens [25,26]. However, unlike ATRA/As₂O₃ which could cleave/degrade the specific fusion proteins PML-RARα resulting in differentiation of APL cells, treatment with CoCl₂ does not modulate PML-RARα, suggesting that the differentiation induction effect of hypoxia and hypoxia-mimetic agents might exist in other leukemic cells. Indeed, the experiments indicated that hypoxia and hypoxia-mimetic agents could also differentiate other subtypes of AML cells such as U937 in a similar manner. Of importance, CoCl₂ induces primary leukemic cells from some AML patients to undergo differentiation, which greatly supports the results observed in the cell lines. Furthermore, non-toxic concentrations of CoCl₂ and DFO each potentiate the growth-arresting effect and differentiation induction of low-dose As₂O₃ in APL cell line NB4 cells [27]. In parallel, the differentiation induction by these treatments is accompanied by the increase of HIF-1α protein and its DNA-binding activity, which suggests that HIF-1 may play an active role in the differentiation of AML cells. Of note, higher concentrations of CoCl₂ and DFO trigger leukemic cells to undergo apoptosis through mitochondrial-dependent and HIF-1α-independent mechanisms [28].

Based on these *in vitro* findings, we investigated the *in vivo* effects of hypoxia on AML mice [29]. For this set of experiments, leukemic blasts from PML-RARα transgenic mice were injected into the tail vein of 7–10-week-old syngenic FVB-NICO (FVB/N) mice after sublethal irradiation totaling 4.5 Gy and the leukemic mice were then housed in a hypoxic chamber equivalent to an altitude of 6000 m for 18 h each consecutive day. Results showed that the survival time of leukemic mice is significantly prolonged with the incubation of intermittent hypoxia while normal mice keep alive under intermittent hypoxia for 60 days without any evidence for hypoxic damage. More importantly, intermittent hypoxia reduces leukemic cell infiltration by inhibiting the proliferation without apoptosis induction and inducing differentiation of leukemic cells in the peripheral blood, bone marrow and peripheral tissues such as spleen and liver, with sharp decrease in monomorphic, immature, promyelocyte-like cells and emergence of a percentage of mainly ring-like terminally differentiated cells.

Moreover, there are several other reports to substantiate our observations. For example, differentiation of normal human CD34+ progenitors cells along all the erythrocytic, megakaryocytic and granulocytic pathways is accelerated in low versus high oxygen tension conditions and murine 32Dcl3 myeloid cells also show accelerated granulocytic differentiation in low oxygen tension in response to granulocyte colony-stimulating factor, which is inhibited by the expression of TEL-ARNT fusion protein [30]. Under low oxygen tension, bone marrow stromal cells (MSCs) exhibit growth-

arrest and differentiation behavior changes. When cultured in adipogenic medium, there is a 5–6-fold increase in the number of lipid droplets under hypoxic conditions compared with that in normoxic culture [31]. Taken together, these findings underscore the differentiation induction of hypoxia or hypoxia-mimic agents in AML cells, which is possibly of significance to explore clinical potentials and novel target-based drugs for differentiation therapy of leukemia.

3. The role of HIF-1α in AML cell differentiation

There is a consistence between the differentiation induction induced by hypoxia/hypoxia-mimic agents and the accumulation of HIF-1α protein under these treatments, which is the master transcriptional factor in cellular response to hypoxia [25,26]. In contrast, while nitric oxide (NO) donor 3-morpholinosydnonimine (SIN-1) abrogates CoCl₂-triggered HIF-1α accumulation and the HRE-binding activity, it significantly antagonizes CoCl₂-induced differentiation of AML cells. Meanwhile, metavanadate antagonizes DFO-induced growth-arrest and differentiation with the inhibition of HIF-1α protein stabilization in leukemic cells [25,32]. In addition, Zhong et al. reported that nuclear expression of HIF-1α protein is heterogeneous in human malignant cells under normoxic conditions [33]. All of these suggest a potential role of HIF-1α protein in leukemic cell differentiation.

To confirm the role of HIF-1α in AML cell differentiation, HIF-1α-expressing vector and empty vector were transfected into U937 cells, the results indicated that with decreased proliferative ability, some maturing cells can be observed under microscope and CD11b⁺ cells% is significantly higher in HIF-1α-transfected cells than those in empty vector-transfected cells [26]. To provide more direct evidence, we generate myeloid leukemic U937T transformants, in which HIF-1α is tightly induced by tetracycline withdrawal [34]. The results showed 3–4 days after the induction of HIF-1α, the proliferation is substantially slowed down and the G1-S phase transition is blocked. More importantly, HIF-1α induction directly triggers myeloid leukemic cells to undergo differentiation, as determined by differentiation-related morphological changes, increased CD11/NBT reduction, and expressions of differentiation signatures such as neutrophil cytosolic factor-1 (NCF1), interleukin-1 receptor antagonist (IL1RN) and secreted phosphoprotein 1 (SPP1). Furthermore, two pairs of shRNAs specifically against HIF-1α mRNA were respectively transfected into the parental U937 cells with negative shRNA as a control [34]. Under the effective interference of HIF-1α protein, CoCl₂ and 2% O₂-induced differentiation of U937 cells is remarkably inhibited, indicating the direct contribution of HIF-1α to hypoxia and CoCl₂-induced differentiation of myeloid leukemic cells.

On the other hand, it is reported that HIF-1α protein could be accumulated in differentiating U937 macrophages in normoxia [35]. Hence, we next investigated whether or not it is associated with the differentiation effect triggered by other

differentiation induction agents, choosing ATRA as a model [36]. To our surprise, the results showed that ATRA rapidly accumulates endogenous and inducible expressed or CoCl₂-stabilized HIF-1 α protein of leukemic cells under normoxic oxygen tension. More importantly, suppression of HIF-1 α expression by specific shRNAs partially but significantly represses while conditional HIF-1 α induction and HIF-1 α -stabilizing CoCl₂ treatment greatly enhances ATRA-induced leukemic differentiation. These observations indicate that as a protein rapidly responsive to ATRA, HIF-1 α does exert a role in the differentiation induction of ATRA on AML cells which provided a new signal pathway with clinical potential for ATRA-induced leukemic differentiation and further supports the role of HIF-1 α protein in leukemic cell differentiation. In consistence, Kim et al. also found that tiron, a widely used anti-oxidant and non-toxic chelator to alleviate an acute metal overload, causes HL-60 cells to induce differentiation-related alterations such as the increase in CD11b and CD14 expression or chromatin condensation through increasing HIF-1 α expression [37].

4. Interaction of HIF-1 α with C/EBP α and Runx1

As we confirm the role of HIF-1 α in leukemic cell differentiation, in the next step we dedicate to unravel the underlying mechanism. It is well known that HIF-1 α protein exerts its role in a series of biological processes through the transcriptional activation of its target genes, which requires the heterodimerization with HIF-1 β . Hence, we first determine whether HIF-1 α -mediated differentiation involves the transcriptional activity of HIF-1 α protein. To this end, two effective pairs of shRNAs specially against HIF-1 β mRNA were transfected into the parental U937 cell line. While the suppression of HIF-1 β protein by shRNAs damages the transcriptional activity of HIF-1, which is evidenced by the inhibition of HIF-1 target genes, 50 μ M CoCl₂ or 2% O₂ treatment for 6 days still induces differentiation of parental U937 cells to the same degree as that in the negative control transfection. Similarly, the silencing of HIF-1 β protein does not impinge on HIF-1 α induction-triggered differentiation after tetracycline withdrawal. Thus, HIF-1 β is not necessary for hypoxia/HIF-1 α -mediated myeloid cell differentiation [34].

On the other hand, our results showed that compared with other leukemic cells, the differentiation induction effect of hypoxia/CoCl₂/DFO is less obvious in the Kasummi-1 cell, which expresses a high level of the chromosomal translocation *t*(8;21)-generated leukemogenic AML1-ETO fusion protein [26]. As the fusion genes are reported to exhibit the inhibitory effect on the differentiation of AML cells induced by a wide spectrum of differentiation-inducing agents [38], we extend our study to investigate the potential inhibitory effect of AML1-ETO expression on hypoxia/CoCl₂/DFO-induced leukemic differentiation. For this purpose, U937-A/E 9/14/18 cells were incubated with ponasterone A, which induced AML1-ETO expression in

the cells in a time-dependent manner [39–44]. While DFO at 5 μ M and 10 μ M induces U937-A/E 9/14/18 cells to differentiate in the absence of ponasterone A, the addition of ponasterone A with inducible expression of AML1-ETO significantly inhibits DFO-induced differentiation in the cells, which indicates that AML1-ETO expression blocks DFO-induced leukemic differentiation. Unexpectedly, the treatment with ponasterone A increases HIF-1 α mRNA time-dependently in U937-A/E 9/14/18 cells, but it fails to stabilize HIF-1 α protein in the alone treatment alone while elevating DFO-accumulated HIF-1 α protein [26].

Combining the observations that the suppression of HIF-1 β does not influence hypoxia/HIF-1 α -mediated differentiation and the contradictory discovery that AML1-ETO expression blocks DFO-induced differentiation but potentiates HIF-1 α protein expression in combination with DFO, we hypothesize that HIF-1 α induces leukemic differentiation by regulating some other differentiation-related factors independent of its transcriptional activity and inhibited by the AML1-ETO fusion protein. As it is reported that AML1-ETO expression results in the inhibition of C/EBP α expression [45], in the next step we investigated the potential role of C/EBP α in HIF-1 α -induced differentiation. To this end, we examined whether HIF-1 α interacts with and affects the transcriptional activity of C/EBP α [26,46]. Co-immunoprecipitation indicated that not only ectopically but also endogenously expressed HIF-1 α protein interacts with C/EBP α . GST pull-down assay proposed that the protein–protein interaction is direct and demonstrated that C/EBP α competes with HIF-1 β for direct binding to HIF-1 α protein. The functional analysis such as EMSA, chromatin immunoprecipitation, luciferase assay and the expression of HIF-1-targeted genes showed that HIF-1 α protein enhances transcriptional activity of C/EBP α while C/EBP α significantly inhibits the DNA-binding ability and transcriptional activity of HIF-1 α . The inhibition of HIF-1 function leads to the reduced expression of VEGF, inhibiting angiogenesis to form a hypoxic microenvironment and thus for leukemic cell differentiation, which provides new evidence of the anti-tumor potential of C/EBP α . Of note, Kim et al. found that tiron also increases HIF-1 α protein and thus the activity of C/EBP α [37].

Considering that C/EBP α , as one of the most important transcriptional factors in hematopoiesis, is always absent in most solid tumors, we extend our study to other important transcriptional factors particularly expressed in hematopoietic system, such as PU.1 and Runt-related protein 1 (Runx1, also known as acute myeloid leukemia-1, AML1) which are also reported to play a role in angiogenesis [47]. The results showed that ectopically expressing Runx1 protein directly interacts with HIF-1 α protein to a degree, as determined by LacO array, Co-IP and GST pull-down assay, and such an interaction can also be detected in endogenously expressed Runx1 and CoCl₂-stabilized HIF-1 α protein. Runx1 inhibits transcription-dependent function of HIF-1 as over-expression of Runx1 suppresses DNA-

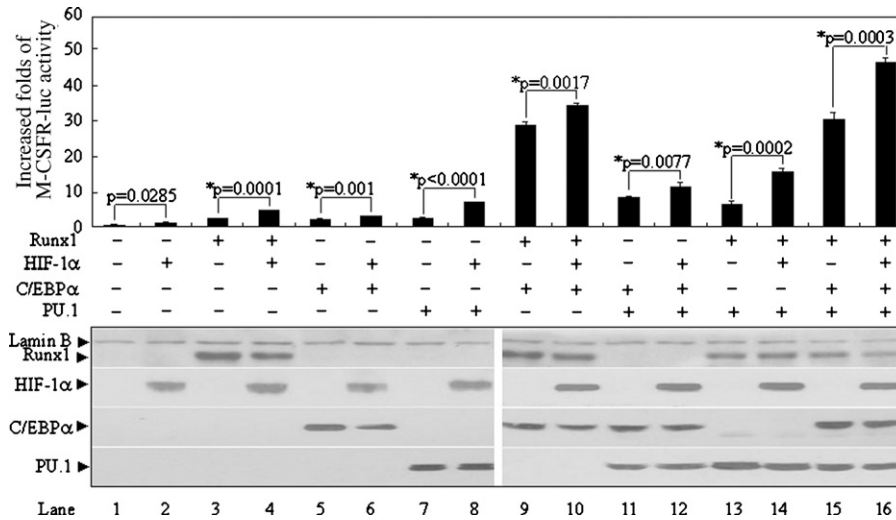


Fig. 1. HIF-1α expression enhances the transcriptional activities of PU.1, C/EBPα and Runx1. 293 cells were transfected with plasmids as indicated in the bottom, together with pM-CSFR-Luc and pSV40-Renilla. Twenty-four hours later, western blots were performed to confirm the effective transfection. The relative M-CSFR-Luc activity was normalized by pSV40-Renilla, and folds of increases against lane 1 were calculated. Symbol* represents p values of less than 0.01 between the lanes indicated.

binding and transcriptional activity of HIF-1 protein with decreased expression of HIF-1-targeted genes, while abrogation of Runx1 expression by specific shRNAs significantly increases transcriptional activity of HIF-1 protein; vice versa, HIF-1α enhances DNA-binding ability and transcriptional activity of Runx1 protein [48].

It has been known that M-CSFR is also synergistically regulated by C/EBPα, Runx1 and PU.1, which are crucial for normal myeloid hematopoiesis [49]. Hence, in the next phase of analysis, we attempted to understand whether HIF-1α impinges on transcriptional activity of PU.1 and synergy of these three transcriptional factors. As for this, PU.1, C/EBPα, Runx1 and HIF-1α, alone or their combination, together with pM-CSFR-Luc plasmids, which contains

binding sites for PU.1, Runx1 and C/EBPα, were transfected into 293 cells effectively. The results showed that alone transfection of HIF-1α, Runx1, C/EBPα and PU.1 (lanes 2, 3, 5 and 7, respectively, Fig. 1) increased transcriptional activity of M-CSFR promoter. Also, combination of HIF-1α/Runx1 (lane 4, Fig. 1), HIF-1α/C/EBPα (lane 6, Fig. 1), Runx1/C/EBPα (lane 9, Fig. 1), C/EBPα/PU.1 (lane 11, Fig. 1), Runx1/PU.1 (lane 13, Fig. 1), and Runx1/C/EBPα/PU.1 (lane 15, Fig. 1) also synergistically increased M-CSFR-Luc activity. More intriguingly, such a synergy could also be seen between HIF-1α and PU.1 (lane 8, Fig. 1). To our excitement, HIF-1α also remarkably potentiated the transcriptional synergy of Runx1/C/EBPα (lane 10, Fig. 1), C/EBPα/PU.1 (lane 12, Fig. 1), Runx1/PU.1 (lane

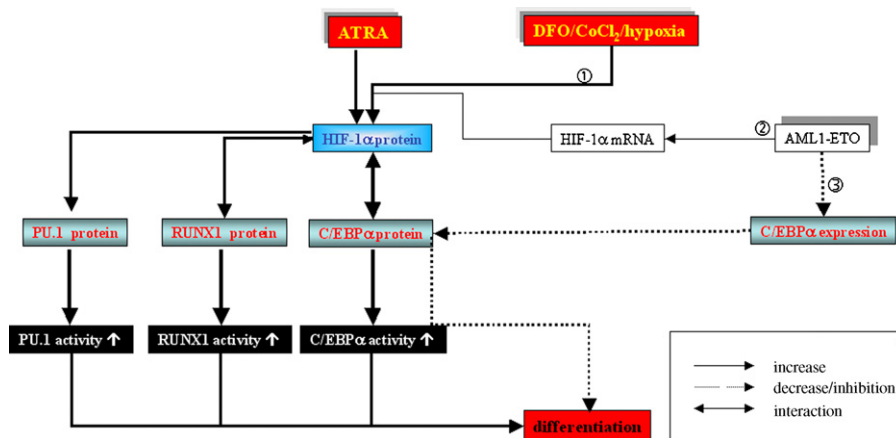


Fig. 2. A recapitulation to describe the mechanism of hypoxia-induced leukemic differentiation. ① Hypoxia and hypoxia-mimic agents triggers leukemic differentiation with the accumulation of HIF-1α protein, which physically interacts with and enhances the transcriptional activities of C/EBPα and Runx1, the downstream effectors of HIF-1α protein to finally induce leukemic differentiation. Also, HIF-1α increased the transcriptional activity of PU.1. Of note, HIF-1α protein contributes to ATRA-induced myeloid leukemic differentiation. ② AML1-ETO increases the mRNA level of HIF-1α but fails to stabilize HIF-1α protein ③ AML1-ETO suppresses the expression of C/EBPα as well as its transcriptional activity. Accordingly, in the presence of AML1-ETO, the leukemic differentiation is partially but significantly inhibited.

14, Fig. 1), and Runx1/C/EBP α /PU.1 (lane 16, Fig. 1) on M-CSFR-luc activity. These findings could also be confirmed in Cos-7 cells.

Finally, we investigated the potential contribution of C/EBP α , Runx1 and PU.1 on hypoxia/HIF-1 α -induced differentiation [34,36]. Thus, shRNAs specifically against C/EBP α , Runx1 or PU.1 were transfected into leukemic U937T^{clone} cells, together with a negative vector transfection as a control (NC). These shRNAs effectively reduce expressions of their cognate targeted genes with no influence of HIF-1 α protein induction after tetracycline withdrawal. More intriguingly, the reduced C/EBP α , Runx1 or PU.1 expression significantly inhibits HIF-1 α induction-triggered differentiation in the U937T^{clone} cell line in the absence of tetracycline, as determined by CD11 expression. Such inhibition can also be observed in CoCl₂-induced differentiation in the cell line in the presence of tetracycline. By the way, differentiation synergy of ATRA and HIF-1 α induction can also be significantly inhibited by the suppression of expressions of these hematopoietic transcriptional factors.

5. Conclusion

Taken together, our results put forward with the notion that hypoxia/hypoxia-mimic agents/HIF-1 α can trigger differentiation in myeloid leukemic cells, in which, HIF-1 α plays a critical role independent of its transcriptional activity while C/EBP α and Runx1 act as the effectors downstream to HIF-1 α protein through direct interactions to enhance their transcriptional activities. Also, HIF-1 α contributes to ATRA-induced leukemic differentiation (Fig. 2). These discoveries would shed new insights for understanding mechanisms underlying leukemogenesis and understanding new therapeutics for differentiation induction of AML.

Albeit, some directions still deserve further investigation. Take the clinical potential of hypoxia/hypoxia-mimic agents for example, it may be of great interest to set the leukemic patients on Qinghai-Tibet Plateau which has an altitude of thousands of miles with lower oxygen tension and to observe whether their survival time is longer than those under the same treatment in the places with lower altitude. In addition, other chelators which might act as hypoxia-mimic agents to stabilize HIF-1 α protein deserve further exploration. Furthermore, as we figure out C/EBP α and Runx1 as the effectors downstream to HIF-1 α protein, some other factors might also link HIF-1 α protein to leukemic differentiation due to the network of the hematopoietic regulation. Actually, we exert the comparative proteomic analysis of hypoxia/CoCl₂-treated and untreated human leukemic U937 cells by 2-DE coupled with MALDI-TOF/TOF MS/MS [50] and have identified some proteins regulated by hypoxia and CoCl₂ treatment, for example, N-myc downstream regulated gene 1 (NDRG1), a putative differentiation-related gene. These would provide new clues for uncovering mechanisms by which leukemic cells respond to hypoxia.

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