Current Understandings of Myeloid Differentiation Inducers in Leukemia Therapy

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Keywords

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Abstract

Differentiation therapy using all-trans retinoic acid for acute promyelocytic leukemia (APL) is well established. Several attempts have been made to treat non-APL, AML patients by employing differentiation inducers, such as hypomethylating agents (HMAs), and low-dose cytarabine (Ara-C) (LDAC), with encouraging results. Other than HMAs and LDAC, various inducers of myeloid cell differentiation have been identified. This review describes and categorizes these inducers, which include glycosylation modifiers, epigenetic modifiers, vitamin derivatives, cytokines, and chemotherapeutic agents. Some of these inducers are currently being used in clinical trials. I highlight the potential applications of glycosylation modifiers and epigenetic modifiers, which are attracting increasing attention in their use as differentiation therapy against AML. Among the agents described in this review, epigenetic modifiers seem particularly promising, and particular attention should also be paid to glycosylation modifiers. These drugs may signal a new era for AML differentiation therapy.

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Introduction

Differentiation therapy using all-trans retinoic acid (ATRA) for acute promyelocytic leukemia (APL) was established in the 1990s and has dramatically improved the clinical outcome of APL. For non-APL, intensive chemotherapy with allogeneic stem cell transplantation is a standard therapy, whereas the optimization of intensive chemotherapy regimens and the use of nonmyeloablative regimens are alternative choices for older patients. Several attempts have been made to improve antileukemic activity in these patients by employing hypomethylating agents (HMAs) with low-dose cytarabine (Ara-C) (LDAC), with encouraging results [1]. These agents mainly serve as differentiation inducers [2, 3].

In this review, I attempt to summarize recent findings about inducers of myeloid differentiation. These include glycosylation modifiers, epigenetic modifiers, micro (mi) RNAs, vitamin derivatives, cytokines, and chemotherapeutic agents. Of these, glycosylation and epigenetic modifiers are attracting particular attention because hematopoietic malignancies have tumor-specific glycosylation and epigenetic modifications that can be used as tumor targets. These inducers may enable the development of efficient differentiation therapy against non-APL, AML.

Fig. 1. The change of cell surface glycosylation and its effect on myeloid differentiation.

Glycosylation Modifiers

Cossu et al. [4] previously reported that the synthesis of high-molecular-weight glycopeptides is a property of human myeloid leukemia cell lines, including HL-60 cells, and that the proportionate decrease in the synthesis of these large glycopeptides is part of monocytic differentiation. Several reports describe differences in cell surface glycosylation changes that occur during hematopoietic cell differentiation, suggesting that glycosylation modifications may play a role in differentiation [5, 6]. The change of cell surface glycosylation and its effect on myeloid differentiation, discussed in this section, is summarized in Figure 1.

Statins were developed to lower cholesterol and triglyceride levels. They act by blocking 3-hydroxy-3-methylglutaryl coenzyme A reductase, which is a rate-limiting step in the mevalonate pathway [7]. This pathway produces dolichol, which is responsible for the co-translational transfer of oligosaccharides to nascent polypeptides that undergo N-linked glycosylation [8]. Interestingly, statins, atorvastatin, and fluvastatin were found to be potent inducers of cell differentiation and apoptosis of the APL cell line, NB4 [9]. This statin-dependent leukemic cell differentiation was found to require activation of the c-Jun NH2-terminal kinase pathway [10] and protein kinase C δ pathways [11].

The most widely occurring cancer-associated changes in protein glycosylation are increased sialylation, an increased number of branched-glycan structures, and the overexpression of core fucosylation [12]. A dramatic increase in cell surface alpha2,6 sialylation was previously reported during the late stage of myeloid maturation [13]. This is restricted to specific glycoproteins including CD11b and CD18. Changes in mature bone marrow myeloid cells are associated with reduced cell binding to fibronectin and cultured bone marrow stroma, which may play a role in the myeloid cell release from the bone marrow.

Notch-dependent control of myelopoiesis has been shown to be regulated by fucosylation [14]. FX is a GDP-4-keto-6-deoxy-mannose-3, 5-epimerase-4-reductase, which is a rate-limiting enzyme for GDP-fucose synthesis. FX knockout mice have a myeloproliferative phenotype and are conditionally deficient in cellular fucosylation through the loss of Notch-dependent signal transduction, which suppresses myeloid differentiation, in myeloid progenitor cells. FLT3 is a type III receptor tyrosine kinase that plays a pivotal role in hematopoietic cell proliferation and differentiation [15]. My group recently discovered that the deletion of core fucosylation by fucosyltransferase 8 (FUT8) knock out in the wild-type FLT3 receptor resulted in the potent activation of its downstream pathway [16]. FUT8 KO led to dimerization of the

FLT3 receptor in the absence of FLT3 ligand, leading to the factor-independent growth of Ba/F3 cells, a mouse interleukin-3-dependent hematopoietic cell line. Because the activation of FLT3, by internal tandem duplication mutation-induced myeloproliferative disease in a transgenic mouse model [17], we discovered that the deficiency of fucosylation, which activates FLT3, has the potential to function in myeloid cell proliferation. We further revealed that the combination of fucosylation inhibitor 2-fluorofucose, with FLT3 inhibitor PKC412, efficiently suppresses the growth of FLT3 expressed Ba/F3 cells [16]. To date, several fucosylation inhibitors have been developed [18–20], and there are many good examples of combination therapy with FLT3 inhibitors for AML [21–23]. Therefore, the modulation of FLT3 glycosylation, combined with FLT3 inhibition, could provide ideas for the development of new therapies for FLT3-mediated leukemias.

Modification of granulocyte colony-stimulating factor (G-CSF) receptor at W318 by C-mannosylation during myeloid differentiation [24] is required for myeloid cell differentiation through activation of the Janus kinase-signal transducer and activator of transcription (STAT) pathway. Previously, the O-GlcNAcylation of STAT5 was found to control tyrosine phosphorylation and oncogenic transcription in STAT5-dependent malignancies [25], while O-GlcNAcylation and tyrosine phosphorylation function together to trigger pYSTAT5 levels and oncogenic transcription in neoplastic cells.

Although the modification of hematopoietic progenitor cell surface glycosylation plays an important role in myeloid cell differentiation, the development of glycosylation-targeted therapy has been limited, possibly because of the complexity of the glycosylation process. However, glycosylation targeting may have the potential to start a new era for leukemia therapy.

Epigenetic Modifiers

Timely myeloid-specific gene expression is required for myeloid cell differentiation, and the lineage-specific transcription factor PU.1 and CCAAT/enhancer-binding protein alpha (C/EBPα) play a pivotal role in this together with DNA methyltransferase (DNMT) and histone deacetylases (HDAC) [26, 27]. Heterozygous somatic mutations in genes encoding epigenetic regulators have been found in all subtypes of myeloid malignancies [28], while epigenetic modifiers such as HDAC inhibitors and HMAs such as 5-aza-2'-deoxycytidine

(decitabine) were shown to induce growth arrest, cell death, and the terminal differentiation of myeloid cells [29, 30]. Already approved related therapeutics include HMAs (azacitidine and decitabine) and isocitrate dehydrogenase (IDH1 or IDH2) inhibitors (ivosidenib and enasidenib) [31]. Kantarjian et al. [32] have reported the efficacy of decitabine, compared to supportive care or LDAC, for the treatment of older (≥65 years) patients with newly diagnosed AML and poor- or intermediate-risk cytogenetics. Since then, the use of HMAs represents an effective alternative for AML patients who are unfit for intensive chemotherapy. In the AML001 study, 488 patients aged 65 years and above with newly diagnosed AML were randomly assigned to receive azacitidine or conventional care regimens (including LDAC, intensive chemotherapy, or best supportive care) and resulted in improved median overall survival of 10.4 months with azacitidine versus 6.5 months for conventional care regimen [33].

The mechanisms of the action of HMAs and HDAC inhibitors are still in debate; however, it was recently reported that the combination of these drugs in AML cell lines results in the massive downregulation of genes coding oncogenes (e.g., *MYC*) and epigenetic modifiers [34]. These genes are often overexpressed in cancer, and this downregulation was associated predominantly with gene body DNA demethylation and changes in chromatin marks acH3K9/27 [34]. Recently, another mechanism of action of decitabine, related to nucleophosmin (NPM) 1, was reported from Gu et al. [35]. NPM1 is one of the most frequently mutated genes in de novo AML (~30% of cases) [36]. Mutant NPM1 represses monocyte and granulocyte terminal differentiation by disrupting myeloid master transcriptional regulators such as PU.1/C/EBPα/RUNX1 collaboration in nucleus, leading to their accumulation in cytoplasm, resulting in the aberrant proliferation and block of differentiation [35]. Mutant NPM1 transforming action can be reversed by pharmacologically directed dosing of decitabine, which depletes the corepressor DNMT1 from C/EBPα/RUNX1 protein interactome [35]. Although several epigenetic therapies have only limited efficacy when used as single agents, combination therapies using conventional chemotherapy that targets AML pathogenesis that exhibit synergistic mechanisms are also undergoing clinical trials [31]. In this context, it was recently shown that the addition of ATRA to decitabine resulted in a higher remission rate and a clinically meaningful survival extension [37]. Another example is venetoclax. Venetoclax is a small molecule inhibitor of the antiapoptotic protein B-cell lymphoma 2 (BCL2).

BCL2 is a mitochondrial protein and functions as an antiapoptotic protein, and AML stem cells are dependent on BCL2 for survival. BCL2 inhibitor was first developed for single agents for AML, but clear synergy with venetoclax and both HMAs and LDAC was identified preclinically [38–41].

Approximately, 15–23% of AML genomes harbor mutations in 1 of 2 isoforms of IDH1 or IDH2 [36]. Leukemic IDH mutations cause changes in genome structure and gene activity, culminating in an arrest of normal myeloid differentiation [42]. Ivosidenib and enasidenib are recently developed IDH1 and IDH2 inhibitors, respectively, and both presented well-tolerated options in the setting of refractory or relapsed AML. Even in elderly and heavily pretreated patients, these 2 agents have shown response rates of 30–40% [43, 44]. Notably, Norsworthy et al. [45] have reported that approximately 19% of the patients with IDH inhibitors were adjudicated as differentiation syndrome. They concluded that differentiation syndrome is a common and potentially fatal adverse reaction of IDH inhibitors and use of standardized diagnostic criteria may aid in earlier diagnosis and treatment. Another important epigenetic change in AML is alterations in histone methylation. This leads to the aberrant silencing of expression of multiple genes [27] involved in tumor suppression and cell cycling, resulting in myeloid maturation arrest. The inhibition of lysine-specific demethylase-1 (LSD1) is a promising novel epigenetic approach for AML therapy. Schenk et al. [46] demonstrated that pharmacologic LSD1 inhibitor, tranylcypromine, restore myeloid differentiation in AML cells. The precise mechanisms of the action were revealed by Ravasio et al. [47] that integration of proteomic/epigenomic/mutational studies showed that LSD1 inhibitors alter the recruitment of LSD1-containing complexes to chromatin, inhibiting the interaction between LSD1 and the transcription factor GFI1. Association of LSD1 with GFI1 has been reported to be critical for maintaining the differentiation block of AML cells [48, 49]; therefore, the dissociation between these 2 factors by LSD1 inhibition plays a role in the induction of myeloid differentiation. Wass et al. [50] recently demonstrated that combination of LSD1 inhibition by tranylcypromine with ATRA can induce differentiation of AML blasts and lead to clinical response in heavily pretreated patients with relapsed/refractory AML with acceptable toxicity. These suggest that the combination of epigenetic modifiers with conventional chemotherapy, or differentiation therapy, may change the use of standard chemotherapy.

microRNAs

Noncoding RNA molecules that regulate DNA transcription and translation include miRNAs, long noncoding RNAs, and circular RNAs [51]. miRNAs are a class of small, noncoding single-stranded RNAs comprising approximately 19–22 nucleotides. They typically inhibit mRNA translation by binding to the 3'-untranslated region of target mRNA [52]. miRNAs are involved in a variety of physiological processes including differentiation [53]. One of the most characterized miRNAs functioning in myeloid differentiation is miR-223, whose expression is regulated by myeloid transcription factor NFI-A and C/EBP α [54]. miR-223 overexpression in APL cell lines enhanced differentiation into granulocytes while inhibiting miR-223 reduced differentiation [54]. However, using a loss-of-function allele in mice, miR-223 was shown to negatively regulate progenitor proliferation and granulocyte differentiation and activation, suggesting that it acts as a fine-tuner of granulocyte production and the inflammatory response [55]. Numerous miRNAs other than miR-223 have been reported as being necessary for myeloid differentiation, including miR-34a [56], miR-24 [57], miR-27 [58], miR-342 [59], and miR-155 [60]. The aberrant expression and function of these miRNAs are important in leukemogenesis.

Vitamin Derivatives

Vitamin A and its analogs, in general, and ATRA, in particular, play a critical role in the differentiation of neutrophils [61]. ATRA exert their antileukemic effects by degrading tumor-specific protein PML-RAR α [62]. Similar to decitabine, as noted before, ATO and ATRA also induce proteasome-dependent degradation of NPM1 mutant leukemic protein, leading to cell growth inhibition and apoptosis in NPM1-mutated AML [63]. Additionally, Vitamin D derivatives such as 1,25-dihydroxyvitamin D3 (1,25D), the hormonal and active form of vitamin D, and its synthetic analogs regulate multiple cell events including cell proliferation, survival, differentiation, and immune responses [64–66]. Although the underlying mechanism of action remains unknown, vitamin K2 has also been reported to induce apoptosis and differentiation [67]. 1,25D induces monocyte/macrophage-like differentiation, and cell cycle arrest through activating various kinase pathways including mitogen-activated kinases, c-Jun NH2-terminal kinases, and p38 mitogen-activated kinases [68]. 1,25D also modulates the expres-

Fig. 2. Myeloid differentiation inducers are described in this review. The reference number of important papers demonstrating clinical significances is shown in roman, while the reference number of basic research papers is shown in italic. Red and blue lines are showing the inducible and suppressive effect on myeloid differentiation, respectively.

sion of several miRNAs [68]. A major limiting factor in the clinical application of 1,25D is the supraphysiologic dose required, which results in systemic hypercalcemia [69]. Vitamin D derivatives have been suggested to have anti-neoplastic properties, but the translation of epidemiological and laboratory findings to the clinic has so far not been successful [68, 70, 71]. However, it may be possible to overcome this using combination therapies of 1,25D with HDAC inhibitors or HMAs [72], thus, highlighting the importance of epigenetic modifiers in differentiation therapy.

Hematopoietic Cytokines and Chemotherapeutic Agents

Many hematopoietic cytokines can trigger the lineage-specific differentiation of leukemia cells, which may have important implications in clinical settings [73]. However, despite numerous laboratory investigations into the differentiation-inducing activities of cytokines, relatively few studies have considered the applications of hematopoietic cytokines in the treatment of leukemia patients [73]. Cytokine therapy implies the stimulation of leukemia cells, but 2 major hematopoietic growth factors, G-CSF and granulocyte macrophage-CSF, are in clinical use to shorten the duration of chemotherapy-induced neutropenia and to prevent infections [74]. The use of these

agents in leukemia was used for 2 major indications: the first, as a primary or secondary prophylaxis of infectious events after therapy in susceptible, older, populations and the second, as a priming strategy to potentiate response to chemotherapy. There is always a theoretical concern that G-CSF may stimulate and mobilize leukemic blasts, and thus, this intervention is approached with caution by most clinicians. Therefore, only few sporadic case reports [75] have described the effectiveness of cytokine therapy for AML treatment. Its therapeutic role remains to be established in more controlled clinical trials.

Various low-dose chemotherapeutic agents have been tested using in vitro differentiation models, including Ara-C [76], antifolates [77], anthracyclines [78], and camptothecin [79]. Among these, LDAC has been used for AML treatment since the 1980s [80]. The mechanisms for differentiation by LDAC remain unclear; however, several findings have been reported. Wang et al. [2] reported that LDAC led to a marked increase of cyclin-dependent kinase inhibitor p21CIP1, which plays a pivotal role in human myelomonocytic leukemia U937 cells. This effect is profoundly impaired in the p21CIP1 antisense stably expressed U937 cells, suggesting that p21CIP1 is playing an important role in the LDAC-mediated U937 differentiation. Chen et al. [81] recently found that LDAC can induce autophagy in AML cells and appeared to play an important role in differentiation and death. They revealed that low-dose (50 nM) cytarabine does not cause

Table 1. Myeloid differentiation agent(s) described in this review

Differentiation agent(s)	Clinical or preclinical	Ref.
<i>Glycosylation modifiers</i>		
Fluvastatin and ATRA	Preclinical	[9]
Atorvastatin and ATRA	Preclinical	[10]
Atorvastatin (or fluvastatin) and ATRA	Preclinical	[11]
<i>Epigenetic modifiers</i>		
HDAC inhibitors	Preclinical	[29]
DNMT inhibitor and TNF- α	Preclinical	[30]
Decitabine	Clinical	[32]
Azacitidine	Clinical	[33]
Decitabine	Preclinical	[34]
Valproate, retinoic acid, and decitabine	Clinical	[37]
5-Azacitidine and BCL2 inhibitor ABT737	Preclinical	[38]
5-Azacitidine and BCL2 inhibitor ABT-199 (venetoclax), ABT737	Preclinical	[39]
Daunorubicin or cytarabine and ABT-199	Preclinical	[40]
IDH1 inhibitor (ivosidenib)	Clinical	[42]
IDH2 inhibitor (enasidenib)	Clinical	[43]
Ivosidenib or enasidenib	Clinical	[45]
LSD1 inhibitor (tranylcypromine) and ATRA	Preclinical	[46]
LSD1 inhibitor (tranylcypromine) and ATRA	Preclinical	[47]
LSD1 inhibitor (OG86)	Preclinical	[48]
LSD1 inhibitor (tranylcypromine and derivatives)	Preclinical	[49]
LSD1 inhibitor (tranylcypromine) and ATRA	Clinical	[50]
<i>Vitamin derivatives</i>		
VVDs	Preclinical	[66]
Vitamin K2 and ATRA	Preclinical	[67]
VVDs and LDAC	Clinical	[70]
<i>Hematopoietic cytokines and chemotherapeutic agents</i>		
LDAC	Preclinical	[2]
Ara-C	Preclinical	[76]
Antifolates	Preclinical	[77]
Anthracyclins	Preclinical	[78]
Camptothecin	Preclinical	[79]
G-CSF, LDAC, and aclarubicin	Clinical	[82]
Low-dose idarubicin	Clinical	[83]
Venetoclax and LDAC or HMA	Clinical	[84]
LDAC with glasdegib	Clinical	[86]

ATRA, all-trans retinoic acid; HMA, hypomethylating agent; LDAC, low-dose cytarabine (Ara-C); HDAC, histone deacetylases; G-CSF, granulocyte colony-stimulating factor; IDH1 or IDH2, isocitrate dehydrogenase; LSD, lysine-specific demethylase; BCL, B-cell lymphoma; DNMT, DNA methyltransferase; VDD, Vitamin D derivative.

apoptosis but induces significant increase in differentiation marker CD11b in monocytic leukemia U937 cells. However, there are dose-dependent increases of apoptotic cells with >500 nM of cytarabine. LDAC induces autophagy, and downregulation of Akt-mTOR pathway is involved in these processes. The CAG regimen, a combination of LDAC, aclarubicin, and G-CSF, has been widely employed in patients with relapsed or refractory AML

[82]. G-CSF stimulates the proliferation of myeloid cells and intensifies Ara-C metabolism. The efficacy of other agents, such as low-dose oral idarubicin treatment, has also been reported [83]. Recently, these low-dose chemotherapeutic treatments have drawn increasing attention, from the development of several promising combination therapies for LDAC. In the pivotal clinical trials evaluating venetoclax, a BCL2 inhibitor described in epigenetic

modifiers section, either in combination with LDAC or with HMA, the rates of complete remission plus complete remission with incomplete hematological recovery were 54 and 67%, respectively, and the median overall survival was 10.4 and 17.5 months, respectively, comparing favorably with outcomes in clinical trials evaluating single-agent LDAC or HMA [84]. Glasdegib, a hedgehog pathway inhibitor, may be used in combination with LDAC for the same indication and improves survival compared with LDAC alone [85]. From randomized phase II, open-label, and multicenter study, it was reported that glasdegib plus LDAC has a favorable benefit-risk profile and may be a promising option for AML patients unsuitable for intensive chemotherapy [86].

Conclusion

To date, numerous differentiation inducers have been reported, but development of their further efficacy is important to expand future applications for differentiation therapies. Attempts to improve the outcome of therapy for AML with HMA or LDAC are, given separately, sometimes in combination with a second agent such as a BCL2 inhibitor venetoclax and the hedgehog inhibitor glasdegib or with novel experimental agents such as IDH inhibitors [32, 33, 38–41, 43, 44, 84–86]. Among the agents described in this review (Fig. 2; Table 1), in my opinion, epigenomic modifiers seem promising [31–33, 37–39, 43, 44, 46], and the combination of these drugs may make an epoch for AML therapy. Particular attention should also

be paid to glycosylation modifiers as combinational agents for leukemia therapy because changes in glycosylation patterns are a unique characteristic of cancer [87], indicating that these inhibitors will work efficiently for hematological malignancies. Further clarifying the mechanism of myeloid differentiation may lead to the development of efficient differentiation therapy for AML.

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Conflict of Interest Statement

The author declares no competing interests.

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Author Contributions

S.T. designed the study, obtained the grants, analyzed the data, and wrote the manuscript.

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