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Is there a role for differentiating therapy in non-APL AML?

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Abstract

Differentiation therapy with all-trans retinoic acid has been a very successful therapeutic strategy in acute promyelocytic leukemia (APL), but the value of differentiation therapy in acute myeloid leukemia (AML) remains to be determined. A number of current treatments, such as tyrosine kinase inhibitors, cytokines, and epigenetic agents, induce differentiation of leukemic cells to some extent, but differentiation is not the main goal of these treatments. Forcing expression of certain transcription factors, such as C/EBP, has also been useful in inducing differentiation in cell lines and in murine models, but an effective way to force expression of these genes in humans is yet to be discovered.

Keywords

acute myeloid leukemia; AML; differentiation; retinoids; PPAR ligands; cytokines; kinase inhibitors; histone deacetylase; methylation; CEBP

Introduction

In 1971, Charlotte Friend developed a cell line eponymously called the Friend cell line that contained a leukemia virus [1]. Friend used dimethyl sulfoxide (DMSO) to transform the Friend cells into red blood cells. This opened the field for a number of researchers to use this cell line to study hemoglobin synthesis and switching of hemoglobin genes. Then, in the myeloid era, three myeloid cell lines were produced that could be induced to differentiate [2–5]. And later, human leukocyte (HL)-60 cell lines were developed that differentiated with exposure to retinoic acid [6,7]. Many other agents can induce differentiation of HL-60 cells, with each agent requiring different molar concentrations for induction (Fig. 1).

Differentiation agents in acute leukemia

Retinoids

The above studies helped to lead to the discovery of the efficacy of retinoids in acute promyelocytic leukemia (APL). APL cells respond to retinoic acid due to the genomic change common to APL (translocation [15;17]), which results in the PML-RAR α fusion

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protein that prevents differentiation. PML-RAR α is targeted by retinoic acid, allowing differentiation of leukemic cells. Retinoids are also used for other differentiation defects, such as some skin problems, but in a randomized study of 13-cis-retinoic acid in myelodysplastic syndromes (MDS) [8], no significant difference occurred between the treatment arm and placebo.

Several studies have utilized all-trans retinoic acid (ATRA) in AML and high-risk MDS. Studies in the United States and the United Kingdom (UK) have not shown an effect of ATRA [9–11], while studies in Germany have shown a benefit to ATRA [12]. In the German study HD98B [12], researchers determined that response to retinoids can be predicted by mutant *NPM1* and wild-type *FLT3* status, but the Medical Research Council AML12 trial in the UK found no effect even with analysis of samples having a *NPM1* mutation [11].

Vitamin D3 is another analog of retinoic acid. Vitamin D3 heterodimerizes with the retinoid X receptor (RXR) and turns on a variety of genes, many of which have antiproliferative and prodifferentiation effects. When vitamin D3 is added to HL-60 cells, the cells differentiate to macrophage-like cells [13], and in vivo activity is good. However, vitamin D causes hypercalcemia [14], so vitamin D analogs that can induce differentiation without hypercalcemia were sought by researchers. Around 100 MDS patients have been treated with vitamin D compounds, but responses have been minor.

PPARγ ligands

Peroxisome proliferator-activated receptor (PPAR)γ ligands bind to the PPAR receptors, which are nuclear hormone receptors, and heterodimerize with RXR. PPARγ ligands have differentiation effects and have been shown to be effective in liposarcomas [15,16], though subsequent studies did not see the same activity. PC3 prostate cancer cell lines can also differentiate in the presence of PPARγ ligands [17], and a large study in prostate cancer showed modestly positive effects [18]. PPARγ ligands also induced differentiation in vivo in myeloid leukemic cells [19], but clinical trials in MDS and leukemias have not shown an effect.

G-CSF

Cloned granulocyte colony-stimulating factor (G-CSF) can also induce differentiation in leukemia cells in vivo [20]. However, evidence suggesting that G-CSF can induce differentiation in fresh leukemia cells is meager. It is more often used to enhance defenses in leukemia rather than cause differentiation. Other cytokines, such as interleukins (IL) 4 and 6, can also induce human myeloid leukemic cell differentiation (Table 1). These cytokines enhance proliferation, are antiapoptotic, and cause differentiation as a natural effect, but are not effective in the in vivo setting.

Congenital neutropenia is a condition associated with a block in differentiation. A proportion of patients with congenital neutropenia have mutation of the G-CSF receptor. When these patients are treated with G-CSF, they experience an increase in mature neutrophils in the peripheral blood. This led to in vitro studies that identified a tyrosine in the distal part of the receptor that was important for induction of differentiation. Elastase is also often mutated in this disease, and apoptosis can account for the lack of differentiation in

the peripheral blood [21]. In congenital neutropenia patients, G-CSF enhances survival and decreases apoptosis but has been associated with a higher incidence of leukemia.

When HL-60 cells were treated with the phorbol diester TPA (12-O-tetradecanoylphorbol 13-acetate), these cells differentiated to macrophage-like cells [22]. TPA causes differentiation within 2 days in almost any leukemic cells from any patient, also becoming macrophage-like in appearance and histochemical staining. Some analogs of TPA have been used in clinical trials, but they have not been very effective in general.

Tyrosine kinase inhibitors

Gefitinib is an epidermal growth factor receptor (EGFR) inhibitor that can induce myeloid differentiation in AML cell lines [23], even though EGFR is not expressed on those cells. This suggests that gefitinib has off-target effects. A microarray expression analysis was performed to identify the signature of myeloid differentiation. An RNA interference (RNAi) library screen was also performed to identify the target of gefitinib that was inducing differentiation. These tests identified a different tyrosine kinase, SYK, as gefitinib's target on AML cells. When AML cells are exposed to gefitinib or the analog R406, the level of phosphorylated SYK decreases, and when SYK is inhibited with small hairpin RNA (shRNA), HL-60 and U937 cells were able to differentiate. The SYK inhibitor also worked in vitro to slow growth of human AML in immunodeficient mice.

A number of tyrosine kinase inhibitors might cause some differentiation. Western blot analysis showed that another EGFR inhibitor, erlotinib, also inhibits phosphorylation of JAK2 and STAT5 [24]. The SYK inhibitor R406 can also induce differentiation in acute lymphoblastic leukemia (ALL) B lymphocytes [25], and the BCR/ABL tyrosine kinase inhibitor imatinib mesylate can cause B-cell differentiation in BCR/ABL-positive B-cell ALL [26].

Epigenetic agents

The discovery by Charlotte Friend that DMSO, a polar planar compound, caused differentiation led to the investigation of other polar compounds, such as hexamethylamine bisacetamide (HMBA) [27]. Suberoylanilide hydroxamic acid (SAHA) is a second-generation polar compound that also functions as a histone deacetylase (HDAC) inhibitor and, in addition, is an inducer of cell differentiation [28]. Chromatin remodeling agents, such as DNA methylation inhibitors (5-azacytidine [azacitidine] and 2-deoxy-5-azacytidine [decitabine]) and HDAC inhibitors, have epigenetic control of gene expression. DNA methylation leads to transcriptional silencing, and inhibitors of DNA methylation lead to demethylation and activation of transcription. HDAC inhibitors acetylate histones and other proteins. Both epigenetically modifying families of drugs can also cause cell-cycle arrest, angiogenesis, immune modulation, and apoptosis. These agents also have some antileukemic and anti-MDS effects, and both azacitidine and decitabine have been approved by the FDA for treatment of advanced MDS.

CEBP

The CCAAT/enhancer binding protein (C/EBP) transcription factors are associated with the differentiation process of a variety of mammalian cells, including hematopoietic cells. In AML, C/EBPα function is abrogated by mutations on either the amino or carboxyl ends of C/EBP. Furthermore, common gene fusion products in AML, such as BCR/ABL and AML1-ETO, can downregulate CEBPα, which impedes differentiation to mature myeloid cells. High levels of *FLT3* ITD mutation can also downregulate CEBPα. In lymphoid leukemias, the *PAX5* gene, a counterpart to C/EBP, is necessary for cells to differentiate from prolymphocytes to mature lymphocytes, and studies have found that 25%–30% of ALL patients have alterations of PAX5 [29,30]. If C/EBPα or C/EBPε expression is genetically induced in myeloid leukemia cells, these cells will differentiate both morphologically and functionally [31,32]. In a murine APL model, forced expression of either C/EBPα or C/EBPε induced differentiation, and overexpression enhanced survival in combination with all-trans retinoic acid [33]. However, ways to force gene expression in humans are unknown, and small molecules to modulate mutant C/EBPα are yet to be discovered.

Conclusions

Leukemic cells gain their growth advantage in part by blocking their own differentiation. Many of the current treatments do induce differentiation to some extent. The ability to reprogram somatic cells to a pluripotent state, through nuclear transfer, cell fusion, or forced expression of a cocktail of genes, is promising [34]. This gives hope that researchers will eventually develop ways to force leukemic cells to differentiate into mature cells, such as neutrophils.

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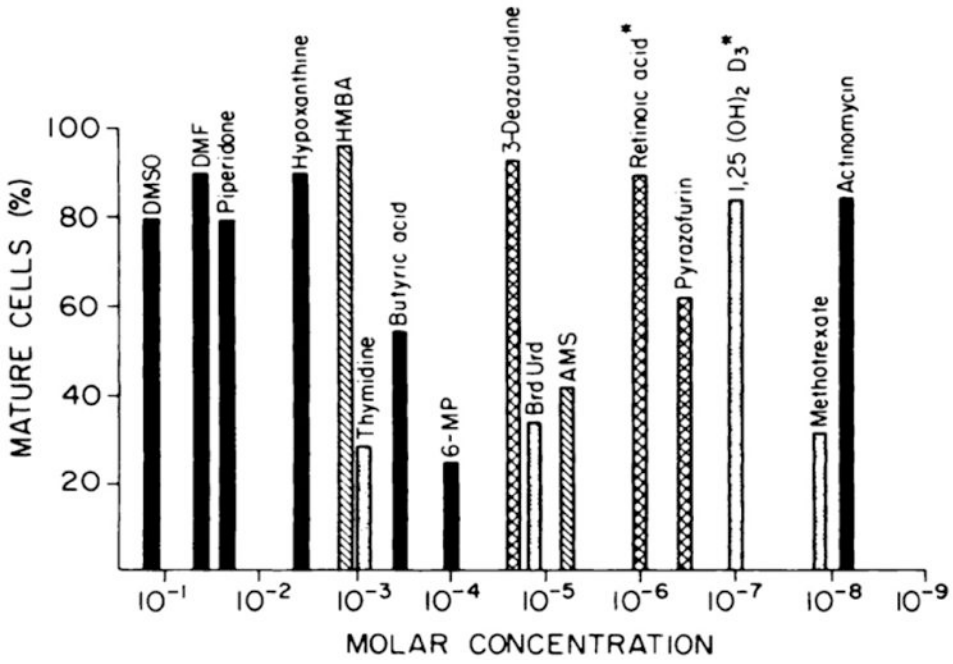

Fig. 1.

Induction of differentiation of HL-60 cell lines [35]. Compounds with solid bars trigger maturation of HL-60 and both murine M-1 myeloid and Friend cells; compounds with striped bars trigger differentiation of HL-60 and Friend cells; compounds with white bars induce differentiation of HL-60 and M-1 cells; and compounds with hash-marked bars only trigger maturation of human HL-60 cells. Abbreviations: DMSO, dimethyl sulfoxide; DMF, dimethyl formamide; HMBA, hexamethylene bisacetamide; 6-MP, 6-mercaptopurine; BrdUrd, bromodeoxyuridine; $1,25(OH)_2D3$, $1,25(OH)_2$ vitamin D3. This research was originally published in *Blood.* Koeffler HP. Induction of differentiation of human acute myelogenous leukemia cells: therapeutic implications. *Blood.* 1983;62:709-21.

Table 1

Cytokines can induce differentiation of human myeloid leukemic cells.

Cytokine	Leukemia lines/Primary leukemia cells	Differentiation lineage
EPO	K562	Erythrocytic
G-CSF	U937	Monocytic
GM-CSF	U937, ML-1	Monocytic
IL-4	U937	Monocytic
IL-6	K562	Megakaryocytic
SCF	AML blasts	Monocytic
SCF or IL-3	AML blasts	Granulocytic
TGF-β	K562	Erythrocytic
TGF-β	HL-60, ML-1, THP-1, U937	Monocytic
TNF-α	HL-60, ML3, U937, AML blasts	Monocytic
IFN-α + GM-CSF	CML mononuclear cells	Dendritic
TGF-β + TNF-α	U937	Monocytic
IL-3 + SCF + TPO	AML blasts	Megakaryocytic
GM-CSF + TNF-α + IL-4	CS-1, KG-1, MUTZ-3, THP-1, AML blasts, CML blasts	Monocytic

Abbreviations: AML, acute myeloid leukemia; EPO, erythropoietin; CML, chronic myeloid leukemia; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; SCF, stem cell factor; TGF, transforming growth factor; TNF, tissue necrosis factor; TPO, thrombopoietin.