Journal of Steroid Biochemistry & Molecular Biology 164 (2016) 59–65

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Novel analogs of 1,25-dihydroxyvitamin D_2 combined with a plant polyphenol as highly efficient inducers of differentiation in human acute myeloid leukemia cells

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ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form 2 September 2015
Accepted 8 September 2015
Available online 10 September 2015

Keywords:
Acute myeloid leukemia
Analogs of 1,25-dihydroxyvitamin D_2
Vitamin D receptor
Cell differentiation
Carnosic acid

ABSTRACT

1α,25-Dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] is known to act as a powerful differentiation inducer in various types of cancer cells, including acute myeloid leukemia (AML) cells. However, supraphysiological concentrations of 1,25$(OH)_2D_3$ required to induce terminal maturation of AML cells can cause lethal hypercalcemia in vivo. Here we characterized the differentiation-inducing effects of novel double-point modified analogs of 1,25-dihydroxyvitamin D_2 [1,25$(OH)_2D_2$], PRI-5201 and PRI-5202 [Pietraszek et al. (2013) Steroids, 78:1003–1014], on HL60, U937 and MOLM-13 human AML cells in comparison with their direct precursors (PRI-1906 and PRI-1907, respectively) and 1,25$(OH)_2D_3$. The results demonstrated the following order of potency for the tested compounds: PRI-5202 > PRI-1907 > PRI-5201 > PRI-1906 ≥ 1,25$(OH)_2D_3$, as determined by measuring the expression of cell surface markers of myeloid differentiation. Particularly, the sensitivity of different AML cell lines to PRI-5201 and PRI-5202 was 3-15-fold and 13-50 fold higher, respectively, compared to that of 1,25$(OH)_2D_3$. Importantly, all the analogs tested at 0.25–1 nM concentrations retained the ability of 1,25$(OH)_2D_3$ to cooperate with the rosemary polyphenol carnosic acid, which strongly potentiated their prodifferentiation activity in a cell type-dependent manner. These synergistic effects were associated with increased induction of the vitamin D receptor (VDR) protein expression. However, surprisingly, carnosic acid was able to significantly enhance only 1,25$(OH)_2D_3$-induced transactivation of the direct repeat 3 (DR3)-type vitamin D response element (VDRE), whereas no such cooperation was seen with 1,25$(OH)_2D_2$ analogs. Furthermore, dose-response analysis revealed that 1,25$(OH)_2D_3$ was more efficacious than the analogs in inducing VDRE activation. This suggests that the superior prodifferentiation activity of the analogs, as compared to 1,25$(OH)_2D_3$, may be due to their potential for enhanced activation of the differentiation-related VDRE(s) that differ from the DR3-type element tested in this study. Collectively, the results demonstrate that the new double-point modified 1,25$(OH)_2D_2$ analogs are much stronger inducers of myeloid differentiation than 1,25$(OH)_2D_3$ and that their efficacy can be further enhanced by combination with plant polyphenols. These combinations warrant their further mechanistic and translational exploration in AML and other types of cancer.

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http://dx.doi.org/10.1016/j.jsbmb.2015.09.014
0960-0760/

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1. Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia in adults and is characterized by a block of terminal differentiation of hematopoietic progenitors at early stages of myelopoiesis. This results in the accumulation of highly proliferative leukemic blasts in the bone marrow which disturbs normal hematopoiesis. Among the patients with newly diagnosed AML, 20–40% individuals do not fully respond to standard therapy with the cytotoxic drugs cytarabine and daunorubicin while 50–70% patients who achieve complete remission are expected to relapse within 3 years, and only about 10% of these patients will survive for 5 years [1]. Furthermore, elderly patients with AML are often ineligible for this treatment due to its toxicity and comorbidities, and outcomes for these patients are particularly poor [2]. Despite a number of experimental drugs developed for the therapy of AML most have failed in clinical trials. Except for gemtuzumab ozogamicin that has been recently withdrawn from the market, no new agent has yet been approved for AML in the last 40 years [3]. Hence the appeal of new sources for novel antileukemic drugs that may effectively and specifically target AML cells.

Differentiation therapy is an alternative AML treatment, based on the induction of leukemic blasts to mature beyond the differentiation block. The differentiation inducer all-*trans* retinoic acid has proven extremely valuable in the treatment of one subtype of AML, acute promyelocytic leukemia (APL) [4]. However, APL accounts for only ~10% of AML, and no differentiation therapy is currently available for other subtypes of AML. Vitamin D derivatives (VDDs), such as 1,25(OH)$_2$D$_3$, the hormonal form of vitamin D, and its synthetic low-calcemic analogs are known to regulate multiple cell events including cell proliferation, survival, differentiation, and immune responses [5,6]. The demonstration of marked antiproliferative and prodifferentiation effects of VDDs on AML cell lines and patient-derived leukemic blasts has suggested a potential therapeutic significance of these agents [6,7]. However, hypercalcemia induced by supra-physiological concentrations of VDDs still remains the major limiting factor for their clinical application [8].

A growing body of research indicates that combination strategy for VDD-based cancer therapy may prove more effective than monotherapy with these agents [6,9]. Preclinical studies in AML cells have shown that VDDs can potentiate growth arrest and cytotoxicity induced by chemotherapeutic agents [10,11]. On the other hand, various compounds, such as differentiation inducers (e.g., ATRA [9,12]), epigenetically active drugs (e.g., 5-aza-2′-deoxycytidine [13]), and anti-inflammatory agents [14,15] were found to enhance VDD-induced cell differentiation. Furthermore, we and others have shown that plant polyphenols, such as carnosic acid [16,17], curcumin [18,19] and silibinin [19,20], markedly potentiate the differentiation-inducing effects of near physiological concentrations of 1,25(OH)$_2$D$_3$ on AML cell lines and patient-derived leukemic blasts. We have also demonstrated that carnosic acid can synergistically enhance cell differentiation induced by low-calcemic 1,25(OH)$_2$D$_3$ analogs [21,22]. The latter findings may have clinical implications for the low-toxic combination differentiation therapy of AML.

We have previously reported the synthesis and evaluation of PRI-1906 and PRI-1907, the analogs of 1,25-dihydroxyvitamin D$_2$

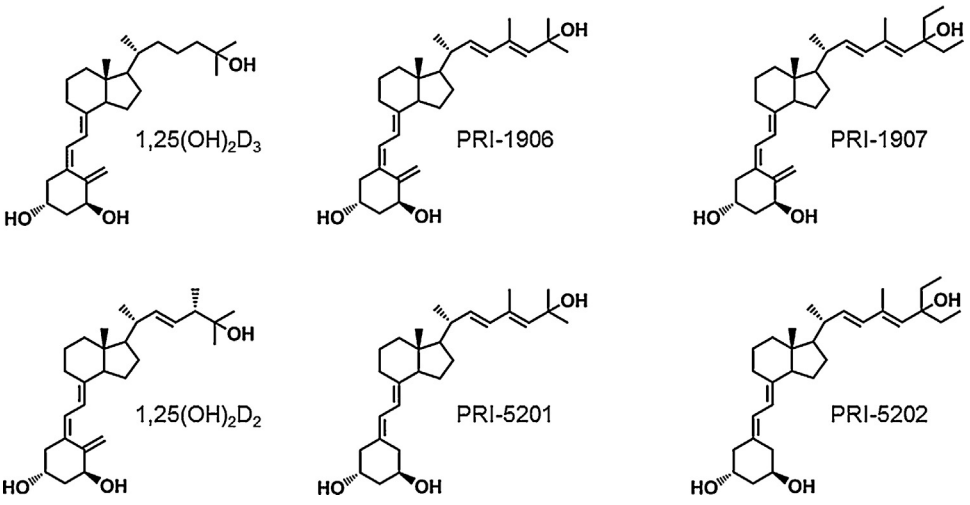

Fig. 1. Structures of the double point-modified analogs (PRI-5201 and PRI-5202) and their respective precursors (PRI-1906 and PRI-1907). Natural active forms of vitamin D$_3$ and D$_2$ are shown for comparison.