Induction of a Functional Vitamin D Receptor in all-trans-Retinoic Acid-induced Monocytic Differentiation of M2-type Leukemic Blast Cells

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ABSTRACT

Different types of acute myeloid leukemia blast cells were induced to differentiate in vitro with all-trans-retinoic acid (ATRA) and vitamin D₃ (VD). M0/M1 leukemic cells are not sensitive to differentiating agents, whereas M3 leukemic cells are induced to undergo granulocytic differentiation after ATRA treatment but are not sensitive to VD. M2 leukemic blast cells behave differently because they undergo monocytic differentiation with both the differentiation inducers. To gain some insight into the maturation of M2-type leukemic cells, we studied the molecular mechanisms underlying monocytic differentiation induced by ATRA and VD in spontaneous M2 blast cells as well as in Kasumi-1 cells (an acute myeloid leukemia M2-type cell line). Our results indicate that ATRA as well as VD efficiently increases the nuclear abundance of VD receptor (VDR) and promotes monocytic differentiation. VDR is functionally active in ATRA-treated Kasumi-1 cells because it efficiently heterodimerizes with retinoid X receptor, binds to a DR3-type vitamin D-responsive element, and activates the transcription of a vitamin D-responsive element-regulated reporter gene. Consistent with these findings, VD-responsive genes are induced by ATRA treatment of Kasumi-1 cells, suggesting that the genetic program underlying monocytic differentiation is activated. The molecular mechanism by which ATRA increases the nuclear abundance of a functional VDR is still unknown, but our data clearly indicate that the M2 leukemic cell context is only permissive of monocytic differentiation.

INTRODUCTION

Myelopoiesis of acute leukemias is characterized by an altered balance between quiescent, cycling, and differentiating cells. AML³ blast cells are, in fact, mainly arrested in the G₁ phase of the cell cycle and are unable to progress spontaneously toward terminal differentiation (1, 2). The growth advantage of leukemic blast cells is therefore achieved mainly through a prolonged survival time, probably caused by a maturation arrest and an inefficient activation of the apoptotic program (3, 4). A unique and specific genetic disorder has been discovered in APL in which the t(15;17) translocation involves the genetic loci of RARα and the promyelocytic leukemia gene (5–8). Patients affected by this type of leukemia can undergo complete remission when treated with ATRA, which induces granulocytic differentiation in vitro and in vivo (9, 10). It is thus evident that the availability of compounds capable of inducing APL blast cells to differentiate and therefore undergo apoptosis provides an opportunity to modify the course of the malignancy.

In a high percentage of the other AML types, genetic abnormalities are extremely heterogeneous and are not specific (11). For example, only 20% of M2-AML is characterized by t (8;21) (12), and a very low percentage of cases carry t(6;9) (13). Moreover, very few cases of AML are characterized by t(3;21) (14). In principle, genetic abnormalities might contribute to the maturation arrest, but no clear correlation can be made between the level of the differentiation block and the genetic aberrations, with the exception of the M3-AML (15).

Furthermore, despite the fact that the vast majority of acute leukemia blast cells express several growth factor receptors (16, 17), they are poorly sensitive to the differentiation activity of the corresponding cytokines in vitro and in vivo; consequently, the therapeutic use of hematopoietic growth factors in AML is controversial (18). Interestingly, cytokine antagonists can block leukemic myeloid cell proliferation due to an autocrine mechanism (19).

Despite the poor evidence that exists on the physiological role of ATRA and VD in normal hematopoiesis, these inducers could be suitable to force the differentiation block in AML blast cells (20, 21).

It is well known that VD and ATRA exert their action by binding to specific nuclear receptors with high affinity. These nuclear receptors regulate gene expression in target cells by binding to specific DNA-responsive elements (VDRE and RARE) via heterodimerization with RXR (22). It has to be pointed out that our previous studies suggest that myeloid precursors at all stages of differentiation are equipped with the machinery required to trigger the VD-dependent genetic program, eventually leading to terminal differentiation.

In other words, they contain VDR protein that is fully active in binding their DNA sites and in promoting transcription (23). The response to VD is therefore likely to depend on steps located downstream of the nuclear receptor/DNA interaction, such as chromatin structure, nucleosome organization, and gene-nuclear matrix interaction.

To gain some insight into the differentiation potential of different types of AML blast cells (M0/M1, M2, and M3), we have investigated the expression levels and function of RARα, VDR, and RXR nuclear receptors (22, 24) and the ability of ATRA or VD to induce terminal granulocytic or monocytic differentiation in vitro (25–27).

Our data suggest that a monocytic differentiation window exists in M2-type blast cells because ATRA and VD can only trigger monocytic differentiation in M2-type Kasumi-1 cells (28) as well as in spontaneous leukemic blast cells.

MATERIALS AND METHODS

Cell Cultures and Differentiation. Blast cell populations were obtained by leukapheresis from five patients with M0/M1-AML, five patients with M2-AML, and three patients with M3-AML before any pharmacological treatment.

All of the blast cells were purified by Ficoll-Hypaque density gradient centrifugation to obtain extremely homogeneous populations (>95% blast cells). The phenotype in each case was defined by morphological, cytchemical (29, 30), immunological (31), cytogenetic, and molecular criteria, at least for the molecular analysis of t(8;21) (32) and t(15;17) translocations (33). The blast...
cell populations were cultured in Iscove’s medium supplemented with 2 mM t-glutamine and 20% heat-inactivated FCS. Kasumi-1 (M2 AML) and HL-60 (M2/M3 AML) cells were cultured in RPMI 1640 supplemented with 15% heat-inactivated FCS and 2 mM t-glutamine. Differentiation was induced in blast cells as well as in Kasumi-1 and HL-60 (34, 35) cells by treatment with either $10^{-8}$ or $10^{-6}$ ATRA (Sigma Chemical Co., St. Louis, MO) or $10^{-7}$ M VD (F. Hoffmann-La Roche, Basel, Switzerland) as described previously (36). The differentiation was monitored by direct immunofluorescence analysis of surface markers (37), such as CD14 (38), CD11b (39), and CD45 (40). Each antibody was incubated directly into the cellular suspension (5–10 $\mu$g/10$^6$ cells) for 20 min at 4°C. The cells were washed twice with PBS and then analyzed by cytofluorometric analysis. Morphology was assessed by cytospin-}
Furthermore, t(8;21) can be detected by RT-PCR in only one of five cases of M2-AML (Fig. 2A, C). C. C., whereas all M3-AMLs were t(15;17) positive (data not shown).

VDR Protein Expression in M0/M1, M2, and M3-AML Blast Cells. Western blot analysis was performed to detect VDR protein in CEs (data not shown) and NEs obtained from M0/M1 (Fig. 3A—C, Lane 1) and M3 blast cell NEs (Fig. 3B, Lane 2), and not in the other types of blast cells studied. The same VDR expression pattern is observed in the other four cases of this type of AML (data not shown).

Fig. 1. Expression of CD45, CD14, and CD11b in M0/M1, M2, and M3-AML cell populations. The histograms show the levels of expression, as evaluated by flow cytometric analysis, of CD45 (hatched bars), CD14 ( ), and CD11b ( ) surface markers in AML M0/M1 (five blast cell populations; A), AML M2 (five blast cell populations; B), and AML M3 (three blast cell populations; C) cells treated with ATRA or VD for 5 days or left untreated. The values are expressed in percentages. The reported results are the average of three different sets of experiments. Variability in each experiment was less than 10%.

Fig. 2. Detection by RT-PCR of RARα, VDR, and RXR mRNAs in AML-M0/M1, M2, and M3 blast cell populations as well as in HL-60 and Kasumi-1 cells. Panel A: Lanes 1–5, five cases of AML-M0/M1; Lanes 6–10, five cases of AML-M2; Lanes 11–13, three cases of AML-M3; Lane 14, negative control performed without the cDNA template. Panel B: Lanes 1, proliferating HL-60 cells; Lane 2, HL-60 cells treated for 12 h with ATRA; Lane 3, HL-60 cells treated for 12 h with VD; Lane 4, proliferating Kasumi-1 cells; Lane 5, Kasumi-1 cells treated for 12 h with ATRA; Lane 6, Kasumi-1 cells treated for 12 h with VD; Lane 7, negative control performed without the cDNA template. RT-PCR products of b2m mRNA are shown at the bottom of both panels. The different genes studied and the size of the amplified fragments are labeled on the left and right side of each panel, respectively.
RARα protein is clearly detected in NEs obtained from untreated M2-AML blast cells (Fig. 3D, Lane 1), and it is not modulated after 12 h of ATRA or VD treatment (Fig. 3D, Lanes 2 and 3). This pattern of RARα protein expression was also observed in NEs obtained from the other types of AML blast cells (data not shown).

Differentiation Effects of ATRA and VD in Kasumi-1 and HL-60 Cells. To better characterize ATRA-induced monocytic differentiation of M2 blast cells, we have compared the differentiation capacity of Kasumi-1 cells derived from the peripheral blood of a patient with M2-AML (28) with the well-known differentiation capacity of HL-60 cells (M3/M3-AML) after 5 days of treatment with ATRA or VD. Morphological analysis performed on differentiated cells shows that only monocytic differentiation can be induced in Kasumi-1 cells with both inducers, whereas HL-60 cells differentiate to monocytes when treated with VD and differentiate to granulocytes after ATRA treatment (data not shown).

This observation was confirmed by flow cytometric analysis of surface marker expression (Fig. 4). In fact, a clear induction of CD11b and CD14 expression (peak 1 and peak 2, respectively) is observed in Kasumi-1 cells after treatment with ATRA (Fig. 4B) or with VD (Fig. 4C), even if a bimodal distribution of CD14/CD11b expression is observed, due to the presence of negative cells. Despite this observation, the majority of Kasumi-1 cells differentiate upon ATRA and VD treatment. HL-60 cells express only CD11b after ATRA treatment (Fig. 4E) and coexpress CD14 and CD11b after VD treatment (Fig. 4F). A high expression level of CD45 is evident in both cell lines before and after treatment with the differentiation inducers (Fig. 4A—F, peak 3).

Fig. 3. Western blot analysis of VDR expression in AML-M0/M1, M2, and M3 blast cell populations. The presence of VDR protein was assayed in NEs obtained from AML-M0/M1 (A), M2 (B), and M3 (C) blast cell populations treated for 12 h with ATRA or VD or left untreated. Lane 1, NEs of untreated cells; Lane 2, NEs of ATRA-treated cells; Lane 3, NEs of VD-treated cells. D, RARα expression in NEs obtained from AML-M2 blast cell populations treated for 12 h with ATRA or VD or left untreated. The estimated molecular mass of VDR and RARα is indicated on the right side of each panel.

Fig. 4. Flow cytometric analysis of CD45, CD14, and CD11b expression in Kasumi-1 and HL-60 cells treated with ATRA or VD for 5 days or left untreated. Peak 1 corresponds to the expression of CD11b, peak 2 corresponds to the expression of CD14, and peak 3 corresponds to the expression of CD45. The expression of the markers listed above has been evaluated in proliferating Kasumi-1 cells (A) and in Kasumi-1 cells treated with ATRA (B) or VD (C), in proliferating HL-60 cells (D), and in HL-60 cells treated with ATRA (E) or VD (F).

VDR and RARα Expression in Kasumi-1 and HL-60 Cells before and after 12 h of ATRA or VD Treatment. Western blot analysis reveals immunoreactive RARα bands in the NEs of both cell lines before and after treatment for 12 h with ATRA or VD (Fig. 5A, Lanes 1—6). Thus, ATRA treatment is not crucial for RARα detection. The apparent molecular weight of this nuclear receptor is not homogeneous in the two different cell types. Because RARα is a phosphoprotein, the size variability ranging from M₉ 54,000 to M₉ 58,000 is probably due to different levels of phosphorylation (64). Two main RARα bands are detected in proliferating and ATRA-treated Kasumi-1 cells (Fig. 5A, Lanes 1 and 2), and only one band of M₉ 58,000...
After 12 h of treatment of Kasumi-1 cells with ATRA or VD, VDR is clearly detected in the major complex (Fig. 6B, Lane 8; Fig. 6C, Lane 14) because a consistent decrease in intensity is caused by the VDR mAb (Fig. 6B, Lane 12; Fig. 6C, Lane 15). No supershift of the major complex is evident in Kasumi-1 NEs after VD or ATRA treatment, when they are matched with RARα mAb (Fig. 6B, compare Lanes 8 and 9; Fig. 6C, compare Lanes 17 and 18). After VD and ATRA treatment of Kasumi-1 cells, the NEs showed an increased abundance of RXR because an increased intensity of the supershifted band is detected with the RXR mAb (Fig. 6B, Lanes 10 and 13; Fig. 6C, Lanes 16 and 19).

These data clearly show that in ATRA- or VD-treated Kasumi-1 cells, VDR can heterodimerize with RXR and efficiently bind to the DR3-responsive element. In HL-60 cells, on the contrary, a VDR-containing bandshift complex is observed only after VD treatment (Fig. 6E, compare Lanes 30 and 31) and not upon ATRA treatment (Fig. 6F, compare Lanes 36 and 37).

Our results indicate that VDR-VDRE complex formation is greatly enhanced by VD or ATRA treatment in Kasumi-1 cells, even if we cannot exclude that a ligand-independent complex might be present in untreated cells (Fig. 6A, compare Lanes 5 and 6).

Fig. 6. EMSA of VDR after binding to a DR3-type DNA element in NEs of Kasumi-1 and HL-60 cells. After 12 h of treatment of Kasumi-1 cells with ATRA or VD, VDR is clearly detected in the major complex (Fig. 6B, Lane 8; Fig. 6C, Lane 14) because a consistent decrease in intensity is caused by the VDR mAb (Fig. 6B, Lane 12; Fig. 6C, Lane 15). No supershift of the major complex is evident in Kasumi-1 NEs after VD or ATRA treatment, when they are matched with RARα mAb (Fig. 6B, compare Lanes 8 and 9; Fig. 6C, compare Lanes 17 and 18). After VD and ATRA treatment of Kasumi-1 cells, the NEs showed an increased abundance of RXR because an increased intensity of the supershifted band is detected with the RXR mAb (Fig. 6B, Lanes 10 and 13; Fig. 6C, Lanes 16 and 19).

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Ability of Endogenous VDR to Activate Transcription of a VDRE-dependent Promoter in Kasumi-1 and HL-60 Cells. To examine the transactivation capacity of endogenous VDR and RARα, we transiently transfected Kasumi-1 and HL-60 cells with the reporter constructs pOsteo/CaBP-CAT and pRAREβ2/TK-CAT and then treated them for 48 h with ATRA or VD. As shown in Fig. 7, both ATRA treatment and VD treatment of Kasumi-1 cells cause transcriptional activation of the pOsteo/CaBP-CAT reporter gene (Fig. 7A, Lanes 4 and 6), whereas ATRA treatment but not VD treatment causes the transcriptional activation of pTK/RAREβ2-CAT (Fig. 7B, Lanes 10 and 12).

This observation provides further evidence that in Kasumi-1 cells, ATRA specifically activates transcription through a VDRE just as efficiently as VD. The effect of ATRA on VDRE-dependent transcription is restricted to M2-type blast cells because no transcriptional activation of pOsteo/CaBP-CAT reporter gene (Fig. 7A, Lanes 4 and 6), whereas ATRA treatment but not VD treatment causes the transcriptional activation of pTK/RAREβ2-CAT (Fig. 7B, Lanes 10 and 12).

The pTK/RAREβ2-CAT construct is transactivated quite consistently in HL-60 cells after ATRA treatment, but not after VD treatment (Fig. 7D, Lanes 24 and 22, respectively).

These results suggest that the pOsteo/CaBP-CAT activation observed in Kasumi-1 cells after ATRA treatment is due to a VDR induction.

Even if several reports indicate that a VD-independent transactivation may occur mainly in kidney and intestine epithelial cells (65–67), pOsteo/CaBP-CAT transactivation observed in Kasumi-1 cells after ATRA treatment seems to be more dependent on increased VDR nuclear abundance than on ligand-independent activation, as also demonstrated by VDR protein expression and EMSA experiments.

Expression of ATRA and VD Primary Response Genes in Kasumi-1 and HL-60 Cells. To monitor the ability of ATRA and VD to activate the genetic program underlying monocytic differentiation in the M2 cellular context, we have investigated the expression of VD-responsive genes (Fig. 8) such as HMSE-1, CD14, and hOC by RT-PCR and the expression of genes controlled by a functional VDRE, such as p21(waf1) (51), or RARE, such as E3 (52), IRF-1 (53) and p21(waf1) (68), by Northern blot analysis after different times of ATRA or VD treatment.

Fig. 8 shows that both ATRA and VD treatment of Kasumi-1 cells causes a rapid induction of HMSE-1, CD14, and hOC expression, demonstrating VDR involvement in ATRA-dependent monocytic differentiation of M2-type blast cells. In fact, these VD-responsive genes are not induced in ATRA-treated HL-60 cells. In addition, neither VD treatment nor ATRA treatment is able to modify the expression of RA primary responsive genes, such as E3 and IRF-1, that are constitutively expressed in Kasumi-1 cells, suggesting that no induction of granulocytic differentiation occurs in these cells (data not shown).

The same analysis performed on HL-60 cells shows a rapid induction of E3 and IRF-1 expression after ATRA treatment, as expected. Despite the differential phenotypic effect observed in HL-60 cells...
with ATRA or VD treatment, p21 \(^{\text{waf-1}}\) expression is induced after 1 h by both differentiating agents, suggesting that its expression is not sufficient to induce monocytic differentiation, as suggested recently (51).

The level of the constitutively expressed \(\beta\)2m mRNA was assessed to monitor RNA abundance in all samples (Fig. 8, Lanes 1–6).

**DISCUSSION**

Differentiation of AML blast cells is blocked at different levels of maturation, as demonstrated by the French-American-British classification (29) and the immunological classification (31). Blast cells maintain the capacity to divide, but the rate of cell division decreases progressively as blast cells undergo a series of differentiation transitions. The consequence of this biological behavior is that the vast majority of leukemic blast cells are arrested in the G1 phase of the cell cycle, and, despite the expression of cyclin-dependent inhibitors, never reach spontaneous terminal differentiation (1, 2, 69). Differentiation induction therapy based on treatment with hemopoietic growth factors such as granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, or monocyte colony-stimulating factor is inefficient because blast cells are not responsive to these cytokines, despite the expression of the corresponding receptors (16–18). *In vitro* experiments indicate that these hemopoietic growth factors are more efficient in recruiting blast cells to the proliferative pathway than to the differentiative pathway (70).

Efficacy of differentiation therapy based on ATRA has been demonstrated only in APL blast cells (9, 10, 71). Because differentiation therapy with physiological inducers is a highly attractive approach to control leukemic cell growth (72), we have evaluated the capacity of ATRA and VD to induce myeloid differentiation in different types of AML blast cells.

We found that spontaneous M2-type AML blast cells, independent of the presence of \(t(8;21)\), are induced to monocytic differentiation with either ATRA treatment or VD treatment. Spontaneous M0/M1 blast cells are not responsive to these inducers. M3-type AML blast cells, as already described, are responsive only to ATRA, and not to VD.

Our original observation is that VDR protein is detected after ATRA treatment in the nuclear compartment of M2 cells, but not in the NEs of M0/M1 and M3 cells, suggesting that both ATRA and VD are able to increase the nuclear abundance of VDR in M2-type blast cells.

To gain some insight into the molecular mechanism underlying monocytic differentiation induced by ATRA in M2-AML blast cells, we studied VDR andRAR\(\alpha\) expression and VDR function in two AML cell lines: (a) Kasumi-1 (M2-type cell line); and (b) HL-60 (M2/M3-type cell line). Kasumi-1 cells are representative of spontaneous M2-AML blast cells because only monocytic differentiation is achieved by ATRA or VD treatment (Fig. 4). HL-60 cells are a classical model of myeloid maturation because they differentiate to granulocytes or monocytes when treated with ATRA or VD, respectively (Fig. 4; Ref. 35).

Our results indicate that nuclear VDR protein is detected in Kasumi-1 cells after either ATRA or VD treatment (Fig. 5). Furthermore, VDR is functionally active in this cellular context because it is capable of binding a DR3 oligonucleotide after heterodimerization with RXR (Fig. 6) and activating the transcription of the transfected pOsteo/CaBP-CAT vector (Fig. 7). These results are obtained in HL-60 cells after VD treatment, but not after ATRA treatment. On the other hand, ATRA can transactivate the pTK/RARE\(\beta\)2-CAT vector (Fig. 7) in HL-60 cells and in Kasumi-1 cells, suggesting that RAR\(\alpha\) is functionally active in both cellular contexts.

Recent reports indicate that there is a cross-over of the nuclear signaling pathways of RA and VD, but it is still unclear whether the interplay between the different nuclear receptors can activate different signaling pathways during hematopoietic differentiation (73–75).

The peculiarity of our results is that both ATRA and VD lead to the activation of monocytic differentiation in M2-type blast cells. Furthermore, the described ATRA-induced pOsteo/CaBP-CAT transactivation is also obtained with compounds such as 8-bromo-cyclic AMP (67) and okadaic acid (66, 67), which are known to induce a VD-independent, VDR-mediated transactivation of reporter genes (65). This transcription activation is achieved through a modulation of VDR phosphorylation levels and does not involve a VDR protein expression induction (67), which is, conversely, clearly observed in ATRA-treated Kasumi-1 cells. Moreover, the formation of spontaneous VD-independent complexes between endogenous VDR-RXR heterodimers and genomic DNA can probably be hypothesized only for tissues characterized by high levels of VDR nuclear protein expression, such as intestinal epithelial cells (65). The existence of basal VDR-RXR/DNA interaction complexes is unlikely in hematopoietic cells, in which the detection of significant amounts of nuclear VDR protein is strictly dependent on VD treatment (23). We can thus conclude that VDR-mediated activation of gene expression induced in Kasumi-1 cells by ATRA probably resides on mechanisms that are not strictly related to VD-independent transcription activation of VDR.

The molecular mechanisms by which ATRA induces a functionally active VDR nuclear protein in Kasumi-1 cells and M2 blast cells are still unclear. Transcription of the VDR gene, which is probably RARE dependent (46), is activated by ATRA in both Kasumi-1 and HL-60 cells because a primary VDR transcript is clearly detectable in both the cellular contexts by RT-PCR, suggesting that the ATRA-induced VDR expression observed in Kasumi-1 cells is not strictly regulated at the transcriptional level.

Furthermore, VDR expression studies in spontaneous leukemic blast cells showed a discrepancy between VDR mRNA and protein levels. VDR mRNA is, in fact, detected in all types of AML blast cells before VD treatment (Fig. 2A). VDR protein (Fig. 3), on the other hand, is detected in the nuclear compartment of all myeloid blast cells only after VD treatment and after ATRA treatment of M2-type blast cells. The RAR\(\alpha\) expression pattern is different because both mRNA and protein are detected in untreated AML blast cells, and protein is not significantly modulated in the NEs after ATRA treatment (Fig. 3D). Comparable results were observed in the HL-60 and Kasumi-1 leukemic cell lines (Figs. 2 and 5).

However, these expression studies indicate clearly that VDR protein abundance in untreated leukemic cells is very low, and that after VD treatment, posttranscriptional mechanisms such as protein stabilization (76) or, as described in other systems, increased translation efficiency of compartmentalized mRNA (77) take part in increasing the VDR protein abundance in the nuclear compartment. This VDR posttranscriptional regulation also occurs in M2-type blast cells after ATRA treatment.

Both ATRA treatment and VD treatment of Kasumi-1 cells (Fig. 8) rapidly induce the expression of VD-responsive genes such as HMSE-1, CD14, and hOC. These data clearly demonstrate that VDR is involved in ATRA-dependent monocytic differentiation of M2-type blast cells. Moreover, these VD-responsive genes are not induced in ATRA-treated HL-60 cells. In addition, neither VD treatment nor ATRA treatment is able to modify the expression of RA primary responsive genes, such as E3 and IRF-1, which are constitutively expressed in Kasumi-1 cells, suggesting that no induction of granulocytic differentiation occurs in M2-type blast cells, even if RAR\(\alpha\) is expressed and is functionally active in transactivating the pTK/RARE/\(\beta\)2-CAT construct. The same analysis performed on HL-60 cells
shows a rapid induction of E3 and IRF-1 expression after few hours of ATRA treatment, as expected. Despite the differential phenotypic effect observed in HL-60 cells with ATRA or VD treatment, p21waf-1 expression is induced after 1 h by both differentiating agents, suggesting that its expression is not sufficient to induce monocyte differentiation, as suggested recently (51). These results are consistent with a recent report showing that p21waf-1 expression after ATRA treatment is associated with granulocytic differentiation of HL-60 cells (68).

In conclusion, our data allow us to hypothesize that the M2 cellular context can be considered a differentiation window permissive only of monocytic differentiation because both VD and ATRA differentiation inducers are capable of activating this differentiation program through the induction of a functional nuclear VDR protein.

This observation might open a new therapeutic application of ATRA, even if the response of AML patients (including M2-type AML) to treatment with this differentiating agent remains to be verified.

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