Platelet-Derived Growth Factor Receptor Regulates Myeloid and Monocytic Differentiation of HL-60 Cells

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Abstract
Here, we show that the platelet-derived growth factor receptor (PDGFR) regulates myeloid and monocytic differentiation of HL-60 myeloblastic leukemia cells in response to retinoic acid (RA) and vitamin D3 (D3), respectively. Both RA and D3 decreased the expression of PDGFR-α and PDGFR-β throughout differentiation. When cells were treated with the PDGFR inhibitor AG1296 in addition to RA or D3, signs of terminal differentiation such as inducible oxidative metabolism and cell substrate adhesion were enhanced. These changes were accompanied by an increased extracellular signal-regulated kinase 1/2 activation. AG1296 also resulted in elevated expression of differentiation markers CD11b and CD66c when administered with RA or D3. Interestingly, other markers did not follow the same pattern. Cells receiving AG1296 in addition to RA or D3 showed decreased G1-G0 arrest and CD14, CD38, and CD89 expression. We thus provide evidence that certain sets of differentiation markers can be enhanced, whereas others can be inhibited by the PDGFR pathway. In addition, we found calcium levels to be decreased by RA and D3 but increased when AG1296 was given in addition to RA or D3, suggesting that calcium levels decrease during myeloid or monocytic differentiation, and elevated calcium levels can disturb the expression of certain differentiation markers.

Introduction
The platelet-derived growth factor receptor (PDGFR) has been implicated in a variety of cancers and leukemias. The constitutively active PDGFR-α fusion protein, FIPL1-PDGFR-α, has been identified in idiopathic hypereosinophilia (1). PDGFR-β is constitutively activated when fused with the transcription factor Tel (2) or rapbmin (5) as seen in some cases of chronic myelogenous leukemias (CML). Up-regulation of PDGFs or PDGFR has been found in cancers (4). The importance of PDGF signaling in cancer was illustrated by the successful treatment of CML (5) and dermatofibrosarcoma protuberans (6) by imatinib mesylate (Gleevec), an inhibitor of the PDGFR family, Abl, and Arg tyrosine kinases (7).

PDGFs are mitogenic during early development, driving proliferation of the undifferentiated mesenchyme (8). PDGFs have been described to mediate the proliferation of oligodendrocyte progenitor cells (9) and inhibit the differentiation of 3T3 pre-adipocytes (10) and bone marrow mesenchymal stem cells (11). The role of the PDGFR in hematopoietic differentiation is not clear, but has been reported to enhance the expansion of early, myeloid, and erythroid progenitors (12). Hence, the fact that immature hematopoietic cells are highly proliferative whereas mature cells are typically growth arrested (13) could be, at least in part, mediated by the PDGFR. It is therefore plausible that PDGF negatively impacts differentiation in hematopoietic cells by favoring an undifferentiated, proliferative state. Indeed, leukemias have been suggested to result from a disruption of differentiation of hematopoietic cells (14).

HL-60 cells are human myeloblastic leukemia cells that serve as a model for studying differentiation induction therapy (15). These cells undergo growth arrest and myeloid differentiation in response to retinoic acid (RA) or monocytic differentiation in response to 1,25-dihydroxyvitamin D3 (D3; ref. 16).

In the current study, we aimed to determine the role of the PDGFR in the myeloid and monocytic differentiation of HL-60 cells. To manipulate PDGF signaling, we employed AG1296, a potent inhibitor for PDGFR and its family members, Kit and Flt3 (17), to antagonize serum or PDGF-BB to promote PDGFR signaling. We find that inhibiting the PDGFR enhances various facets of both RA-induced myeloid differentiation and D3-induced monocytic differentiation, including inducible oxidative metabolism, a functional differentiation marker characterizing mature myeloid or monocytic cells. However, other features typical of these induced differentiation programs were diminished, including induced G0 cell cycle arrest. PDGFR dependence has thus provided a means of grouping differentiation markers associated with functional differentiation and distinct markers associated with G0 arrest.

Materials and Methods
Cell culture. HL-60 human myeloblastic leukemia cells were grown in RPMI 1640 supplemented with 5% heat-inactivated fetal bovine serum (both Invitrogen) and 1× antibiotic/antimycotic (Sigma) in a 5% CO2 humidified atmosphere at 37°C. RA and D3 (Sigma) were added from a stock solution in ethanol with a final concentration of 2 and 0.5 μmol/L, respectively. AG1296 (Calbiochem) was added to cell cultures 1 h before RA and D3 treatment at a concentration of 10 or 20 μmol/L from a stock solution (20 mmol/L) in DMSO. Equal amounts of DMSO were added to all treatment groups not receiving AG1296. For experiments using 100 ng/mL PDGF-BB (Antigenix), cells were cultured in 1% serum 24 h before RA and D3 treatment at a concentration of 10 or 20 μmol/L from a stock solution (20 mmol/L) in DMSO. Equal amounts of DMSO were added to all treatment groups not receiving AG1296. For experiments using 100 ng/mL PDGF-BB (Antigenix), cells were cultured in 1% serum 24 h before and during treatment. Experimental cultures were initiated at a density of 0.2 × 10⁶ cells/mL. Selected experiments were confirmed in NB4 cells, which were cultured and treated as described above.

CD11b, CD14, CD38, CD66c, CD89, and PDGFR expression studies. About 0.5 × 10⁶ cells were collected from cultures and centrifuged at 1,000 rpm in a microfuge for 5 min. Cell pellets were resuspended in 100 μL 37°C PBS containing 5 μL of the respective antibody (allophycocyanin-conjugated CD11b, FITC-conjugated CD14, phycoerythrin-conjugated CD38, PDGFR-α, and PDGFR-β; all from BD Biosciences). Following 1 h incubation at 37°C, cell surface expression levels were analyzed with a BD LSRII flow cytometer (BD Biosciences). To stain for CD66c, we used a mouse primary antibody with dual specificity to CD66c and CD66e (Genetex) for 1 h.

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washed with PBS, stained with an Alexa 350–conjugated goat anti-mouse secondary antibody (Invitrogen), and analyzed by flow cytometry. Undifferentiated control cells were used to determine the fluorescence intensity of cells negative for the respective surface antigen. The gate to determine percent increase of expression was set at the highest 5% of the control population. Results given as mean fluorescence intensity were determined by background staining, and a gate to quantify cells staining for pPDGFR was used to determine percent cells expressing CD11b upon RA or D3 treatment alone.

**Measurement of inducible oxidative metabolism.** About 0.5 × 10⁶ cells were collected and pelleted, resuspended in 200 μL 37°C PBS containing 5 μmol/L 5-(and 6)-chloromethyl-2,7′,dichlorodihydro-fluorescein diacetate acetyl ester (H₂DCF, Molecular Probes) and 0.2 μg/mL 12-O-tetradecanoylphorbol-13-acetate (TPA, Sigma). Both H₂DCF and TPA stock solutions were made with DMSO at concentrations of 0.2 mg/mL and 5 mmol/L, respectively. Cells were incubated for 20 min at 37°C before analysis by flow cytometry. Oxidized DCF was excited by a 488-nm laser, and emission was collected through a 505 long-pass dichroic mirror and a 530/30-nm bandpass filter. The shift in fluorescence intensity in response to TPA was used to determine the percent cells, with the capability to generate inducible oxidative metabolites. Gates to determine percent positive cells were set at the highest 5% of control cells not stimulated with TPA.

**Calcium measurement.** Cell cultures (1 mL) were sampled and incubated in 1 μmol/L fluo-3 acetoxyethyl ester (Molecular Probes). After 1 h in 37°C, cells were washed with 37°C PBS. Cells were resuspended in 200 μL 37°C PBS and incubated for another 30 min to allow complete de-esterification of intracellular fluo-3 acetoxyethyl esters. Calcium-bound fluo-3 has an emission maximum of 526 nm that was quantified after excitation with a 488-nm laser and collection through a 530/30-nm bandpass filter by flow cytometry. Mean fluorescence intensity was determined from the entire cell population.

**Extracellular signal-regulated kinase 1/2 phosphorylation assay.** About 0.5 × 10⁶ cells were collected from cultures and pelleted, fixed by 2% paraformaldehyde, and permeabilized using 90% ice-cold methanol. Samples were washed in PBS, stained with allophycocyanin-conjugated antibody specific for mitogen-activated protein kinase (MAPK) p42/44 dually phosphorylated at Thr382 and Tyr385 (Cell Signalling). Flow-cytometric analysis was done following a 1-h incubation period. Fluorescence intensity of control cells was assumed to represent basal levels of pERK. Logic gates defining the percentage of cells exceeding basal pERK levels were set at the highest 5% of untreated control cells.

**Cell adhesion.** To obtain a relative count of adhesive cells, we treated 5-ml cell cultures with RA or D3 in the absence or presence of AG1296. Both H₂DCF and TPA were incubated in 1 μmol/L fluo-3 acetoxyethyl ester (Molecular Probes) and 0.2 μg/mL 12-O-tetradecanoylphorbol-13-acetate (TPA, Sigma). Both H₂DCF and TPA are excited by a 488-nm laser, and emission is collected through a 505 long-pass dichroic mirror and a 530/30-nm bandpass filter by flow cytometry. Mean fluorescence intensity was determined from the entire cell population.

**Cell cycle analysis.** About 0.5 × 10⁶ cells were collected from cultures. The cytotox was removed by resuspending the cell pellet in hypotonic staining solution containing 50 μg/mL propidium iodide (PI), 1 μL/mL Triton-X100, and 1 mg/mL sodium citrate. Cells were incubated for 1 h at room temperature and analyzed by flow cytometry. Doublets were identified by a PI signal width versus area plot and excluded from analysis.

**Fluorescence resonance energy transfer analysis of PDGFR ubiquitination.** PDGFR ubiquitination was determined by fluorescence resonance energy transfer (FRET). Cells were paraformaldehyde and methanol fixed as described above. We used mouse anti-human PDGFR-α or PDGFR-β (BD Biosciences) and rabbit anti-ubiquitin (Abcam) antibodies as primary antibodies. Secondary goat anti-mouse and goat anti-rabbit antibodies were tagged with Alexa 350 and Alexa 430, respectively (both Invitrogen). Specific staining of the PDGFR and ubiquitin was determined by detecting Alexa 350 by 325-nm excitation and collection through a 440/40 bandpass filter and Alexa 430 by 488-nm excitation and collection through a 530/30 bandpass. PDGFR ubiquitination was determined by measuring the energy transfer from Alexa 350 to Alexa 430 reflected by the emission from Alexa 430 collected through a 530/30 bandpass filter after 325 nm excitation. When the fluorescent tags are in a proximity close enough, the donor (Alexa 350) can excite the acceptor (Alexa 430) by emitting light in the range of 430 to 450 nm, causing the acceptor to emit at 530 nm. Background was set using the primary and secondary antibody for PDGFR only and the secondary antibody for ubiquitin.

**PDGFR phosphorylation assay.** HL-60 cells were cultured for 24 h in 1% serum with and without 10 μmol/L AG1296. Samples (1 mL) were taken from the cell culture and stimulated or not with 100 ng/mL PDGF-AB (Antigenix) for 10 min. Cells were paraformaldehyde and methanol fixed. We used a 1:100 dilution for the primary antibodies against pY1021 PDGFR-β (GeneTex) and pY754 PDGFR-α (Cell Signalling), respectively, and a 1:200 dilution of FITC-conjugated anti-rabbit secondary antibody (BD Biosciences). The FITC signal was analyzed by 488 nm excitation and collecting emission through a 505 long-pass dichroic mirror and a 530/30-nm bandpass filter. Cells stained with secondary antibody alone was used to determine background staining, and a gate to quantify cells staining for pPDGFR was set to exclude at least 95% of the background signal.

**Statistical analysis.** Statistical analyses were done using SPSS 11.0 for Windows, Student Edition. Means of treatment groups of interest were compared using the paired-samples t test. All treatment groups were compared using the paired-samples t test. All treatment groups were compared using the paired-samples t test. All treatment groups were compared using the paired-samples t test. All treatment groups were compared using the paired-samples t test.
compared with control cells. The effect of AG1296 was determined by comparing groups receiving RA or D3 with groups receiving RA or D3 plus AG1296. A \( P \) value of <0.05 was considered significant.

**Results**

**Inhibition of the PDGFR increases the expression of CD11b and CD66c.** To investigate the effect of the PDGFR on differentiation markers CD11b and CD66c cells were treated with the inhibitor of the PDGFR, AG1296, 1 h before RA or D3 administration. AG1296 increased the D3-induced CD11b expression from 48.1% to 78.2% (\( P = 0.016 \)). AG1296 also increased the RA-induced CD11b expression from 24.3% to 35.1% (Fig. 1A). After 72 h, AG1296 treatment increased the RA- or D3-mediated expression of CD66c in a dose-dependent manner (Fig. 1B). RA-treated cells with the 20 \( \mu \)mol/L AG1296 had a significant higher expression of CD66c compared with cells receiving RA alone (\( P = 0.025 \)) or RA plus 10 \( \mu \)mol/L AG1296 (\( P = 0.036 \)). D3 plus 20 \( \mu \)mol/L AG1296-treated cells had significantly higher CD66c expression levels when compared with cells receiving D3 alone (\( P = 0.023 \)) or D3 with 10 \( \mu \)mol/L AG1296 (\( P = 0.031 \)). Consistent with the effect of the PDGFR inhibitor AG1296, low (1%) serum accelerated the expression of CD11b (Fig. 1C) compared with treatment groups cultured in 5% or 10% serum. PDGFR, if activated by serum, retards CD11b expression, whereas inhibiting PDGFR enhances CD11b expression. Cells receiving RA in 10% serum had significantly lower CD11b expression compared with RA-treated cells in 1% serum (\( P = 0.048 \)).

**Inhibition of the PDGFR increases the percentage of functionally differentiated cells.** Here, we tested whether inhibiting the PDGFR with AG1296 would also increase the percentage of cells capable of inducible oxidative metabolism in response to RA or D3. Pretreating for 1 h with AG1296 before RA administration increased the percentage of responsive cells from 10.2% to 33.9% (\( P = 0.032 \), Fig. 2A). Likewise, when cells were treated with AG1296 before D3 treatment, 46.3% of cells were capable of producing ROS compared with 27.4% of cells that were treated with D3 alone. In contrast, high serum for 48 h (Fig. 2B) resulted in a decrease in the percentage of cells capable of inducible oxidative metabolism. RA-treated cells in 10% serum had significantly fewer cells capable of producing reactive oxygen species (ROS) compared with RA-treated cells in 5% serum (\( P = 0.043 \)). Cells treated with D3 in 5% serum showed a significant induction of cells capable of producing ROS (\( P = 0.032 \)), whereas cells treated with D3 in 10% serum did not. To test whether the PDGFR was specifically involved in the modified response to RA or D3, we used 100 ng/mL PDGF-BB to activate the PDGFR. At 24 h posttreatment, 29.9% of RA-treated cells were capable of inducible oxidative metabolism, whereas only 8.9% of cells treated with RA and PDGF-BB were capable of inducible oxidative metabolism (Fig. 2C). Similarly, when cells were treated with D3 and PDGF-BB for 24 h, 11.0%
(P = 0.018 compared with D3) of cells were capable of inducible oxidative metabolism compared with 18.1% of cells treated with D3 alone (Fig. 2C).

**AG1296 increases substrate adhesion of myeloid or monocytic HL-60 cells.** Substrate adhesion is another attribute of myeloid/monocytic differentiation of HL-60 cells. Counting four random fields of a Zeiss IM35 microscope using 400× magnification, on average, 22 cells adhered to the cell culture flask when treated with RA only compared with 45 (P = 0.034) when cells were treated with AG1296 and RA (Fig. 2D). Similarly, the number of adherent cells increased from 122 when cells received D3 to 237 (P = 0.030) when cells received AG1296 in addition to D3. Although adhesiveness is a marker of enhanced differentiation, it also contributes to retinoic acid syndrome, a common complication during RA treatment of patients with acute promyelocytic leukemia, which is characterized by leukemic cells adhering to endothelial cells in cardiac and pulmonary tissues (18).

**Inhibition of the PDGFR decreases CD38, CD14, and CD89 expression.** We also investigated the effect of AG1296 on CD14, CD38, and CD89 expression. After 24 h, RA induced CD38 expression in 90.7% cells, whereas pretreatment with AG1296 for 1 h decreased the RA-responsive CD38 expression to 70.7% (P = 0.035, Fig. 3A). Likewise, CD38 expression in response to D3 was reduced from 55.2% to 39.4% (P = 0.040) when the PDGFR was inhibited with AG1296. CD14 expression was also reduced, decreasing from 29.3% for RA-treated cells to 17.3% (P = 0.030 for RA- and AG1296-treated cells). About 70.3% of D3-treated cells expressed CD14 compared with 66.7% of D3- and AG1296-treated cells. Similarly, RA-induced CD89 expression was reduced from 31.6% to 16.1% (P = 0.005), when AG1296 was used in addition to RA. AG1296 also had less effect on D3-mediated CD89 expression. About 54.3% of D3-treated cells expressed CD14 compared with 53.0% of D3- and AG1296-treated cells.

CD89 was not significantly increased by increasing serum (Fig. 3B). However, D3-treated cells in 5% serum had significantly (P = 0.020) higher CD38 expression compared with cells treated in 1% serum, and D3-treated cells in 10% cells had significantly higher CD38 expression compared with both D3-treated cells in 1% serum (P = 0.016) and 5% serum (P = 0.044).

Similarly, PDGF-BB could increase CD38 expression induced by RA from 17.1% to 22.3% (P = 0.013) and induced by D3 from 7.8% to 13.8% (Fig. 3C).

The fact that these markers did not follow the pattern of other differentiation markers indicates a differential effect of the PDGFR on various differentiation markers.

**AG1296 has similar effects on differentiation markers in NB4 cells.** To determine if the effect of AG1296 on differentiation was unique to HL-60 cells, we also tested the expression levels of selected markers in NB4 cells that were treated with RA or D3 plus

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**Figure 3.** Inhibition of PDGFR causes decreased expression of CD14, CD38, and CD89 in HL-60 cells and elicits similar effects on differentiation markers in NB4 cells. PDGFR inhibitor AG1296 (10 μmol/L) decreased the percentage of cells expressing CD38 (after 24 h), CD89 (after 48 h), and CD14 (after 72 h) of RA or D3 treatment (A), whereas increasing serum (1%, 5%, and 10% serum; B) and 100 ng/mL PDGF-BB (C) increased expression of CD38. D, effect of AG1296 (10 μmol/L) treatment on differentiation markers in NB4 cells. CD38 expression was determined 24 h after treatment with RA or D3 in the absence or presence of AG1296 and CD11b, and inducible oxidative metabolism (ROS) was determined 48 h posttreatment. Columns, means of three independent experiments; bars, SE. *, treatment groups that were significantly different from control; #, treatment groups that were significantly different from their respective groups receiving only RA or D3.
or minus AG1296. Similar to results seen in HL-60 cells, AG1296 decreased the RA- or D3-induced expression of CD38 (Fig. 3D). The effect was significant (P = 0.003) in RA-treated compared with RA plus AG1296–treated cells. As in experiments using HL-60 cells, AG1296 increased CD11b expression in RA- or D3-treated cells. D3 plus AG1296–treated cells expressed significantly (P = 0.027) more CD11b compared with cells treated with D3 alone. Similarly, D3 plus AG1296–treated cells also had significantly (P = 0.039) more cells capable of inducible oxidative metabolism compared with cells treated with D3 alone.

AG1296 decreases cells in G0 arrest in response to RA or D3. Terminally differentiated cells undergo growth arrest. Inhibiting the PDGFR with AG1296 suppressed the G1-G0 arrest induced by RA (P = 0.021, RA compared with RA plus AG1296; Fig. 4A) as determined by DNA histograms generated by flow cytometry. Cells treated with RA (P = 0.004), D3 (P = 0.005), or D3 plus AG1296 (P = 0.003) all showed significant G1-G0 arrest when compared with control cells. As for the cell surface differentiation markers, CD14 and CD89, where AG1296 decreased expression in response to RA, AG1296 decreased RA-induced G0 arrest but had only a minimal effect decreasing D3-induced G1-G0.

AG1296 increases ERK1/2 phosphorylation. We aimed to determine the effect of AG1296 treatment on extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation, which has been shown to be an essential driver of differentiation of HL-60. RA plus 10 or 20 μmol/L AG1296 had significantly higher pERK phosphorylation compared with RA alone (P = 0.006 and P = 0.003, respectively). D3 plus 20 μmol/L AG1296 had significantly higher pERK phosphorylation when compared with D3 alone (P = 0.024) or D3 plus 10 μmol/L AG1296 (P = 0.008). AG1296 thus enhanced RA- or D3-induced MAPK activation. To address the counter-intuitive effect of inhibiting PDGFR on ERK1/2, we also evaluated ERK1/2 phosphorylation upon short-term exposure to AG1296. AG1296 did not cause ERK1/2 phosphorylation when applied for short periods ranging from 10 min to 12 h (data not shown), indicating that the increase at 24 h is likely a secondary effect.

Inhibiting the PDGFR increases Ca2+ levels in RA- and D3-treated cells. Increased Ca2+ levels have been described to decrease CD38 (19, 20) but increase CD11b (21) expression. At 48 h RA or D3 posttreatment, Ca2+ was decreased compared with the control group (Fig. 5). In cells that received AG1296 before RA or D3 treatment, Ca2+ levels increased (P = 0.049 RA compared with RA plus AG1296). Although the Ca2+-decreasing effect of RA and D3 was more apparent at later time points (48 and 72 h), we could detect increased Ca2+ in cells treated with AG1296 and RA or D3 as early as 3 h posttreatment. Hence, the higher Ca2+ level may have contributed to the differential effects of AG1296 on differentiation markers, e.g., CD38 and CD11b.

RA and D3 decrease the expression of the PDGFR. Starting at 24 h RA or D3 posttreatment, the cell surface PDGFR expression was reduced. The changes were most significant at 72 h posttreatment when RA reduced PDGFR-α and PDGFR-β (Fig. 6A) to 82.5% and 89.16% of vehicle-treated control cells, respectively. In D3-treated cells, PDGFR-α and PDGFR-β expression was reduced to 42.3% (P = 0.024, D3 compared with control) and 62.24% (P = 0.046, D3 compared with control), respectively. These data support the notion that lowered PDGFR signaling is promoting aspects of the differentiation processes.

PDGFR ubiquitination. Although we could not detect any significant increase in PDGFR-α ubiquitination in cells treated separately with AG1296, RA, or D3, we observed a dose-dependent increase of FRET when RA was used with AG1296 (Fig. 6B). Similarly, we only observed PDGFR-β ubiquitination following simultaneous treatment with RA and AG1296. These data indicate
that the expression of PDGFRs is regulated differently during RA-induced myeloid versus D3-induced monocytic differentiation. At least in the case of RA, the addition of AG1296 induces ubiquitination of the PDGFR.

**PDGFR-α and PDGFR-β phosphorylation.** All cells seemed to have at least some PDGFR-α phosphorylation at Y754 as judged by the fluorescence signal that was distinctly different (no overlap in the flow cytometry histograms) from the background signal obtained with the secondary antibody alone (Fig. 6C). PDGF increased Y754 phosphorylation of PDGFR-α from a relative mean of 272 to 514 (without AG1296) and 398 (with AG1296), respectively.

When analyzing phosphorylation of Y1021 PDGFR-β, we found 42.8% of untreated control cells to stain positive for pY1021 PDGFR-β as determined by scoring cells that were not at least in the upper 5% of the background signal as pY1021 PDGFR-β negative (Fig. 6D). About 10 min of PDGF increased the percentage of pY1021 PDGFR-β-positive cells to 73.2% in cells not treated with AG1296 and decreased the percentage to 36.2% in cells treated with AG1296.
Discussion

Here, we report that the PDGFR regulates RA- and D3-induced phenotypic conversion of HL-60 human myeloblastic leukemia cells. Inhibiting PDGFR with AG1296 caused increased expression of CD11b and CD66ce in response to RA or D3. These cells also had increased ability to produce reactive oxidative species in response to TPA and increased substrate adherence. These findings are in line with previous reports that suggest that PDGFR promotes the undifferentiated, proliferative state of hematopoietic progenitor cells (12). It has also been observed that PDGFR can maintain human embryonic stem cells in an undifferentiated state (22). Consistent with this are our data on decreased PDGFR-α and PDGFR-β expression following RA or D3 treatment. PDGFR expression has been shown to be down-regulated through ubiquitination by the ubiquitin E3-ligase activity of c-CBL following receptor activation (23). Our laboratory observed c-CBL expression to be altered in RA- and D3-treated HL-60 cells. We therefore hypothesized that the decreased PDGFR expression following RA and D3 treatment is due to PDGFR ubiquitination and degradation, possibly mediated by c-CBL. However, RA or D3 treatment did not result in PDGFR ubiquitination. Only cells receiving RA as well as PDGFR inhibitor AG1296 showed PDGFR-α and PDGFR-β ubiquitination. It is unclear why AG1296 did not induce PDGFR ubiquitination in D3-treated cells.

When examining other differentiation markers, such as CD38, CD14, CD89 and G1-G0 arrest, we obtained contrasting results suggesting that the PDGFR pathway is required for these differentiation markers to be expressed. Our data therefore show that certain differentiation markers seem to be positively regulated by PDGFR signaling, whereas others seem to be negatively regulated.

Because calcium had been described to decrease CD38 (22, 23) expression but increase CD11b (24) expression, we also examined calcium levels in our experimental setting. Ca2+ has been proposed to mediate negative feedback regulation of CD38 expression. cADPR, the product of the enzyme CD38, is capable of elevating Ca2+ levels (24), whereas intracellular Ca2+ elevations induced CD38 promoter activation (22) and gene expression (23).

Hence, our findings that Ca2+ levels were decreased during RA and D3 treatment and increased in the presence of AG1296 could cause divergent responses of differentiation markers such as CD38 and CD11b.

The PDGFR is implicated in calcium signaling because the PDGFR can activate PLCγ2, resulting in the generation of diacylglycerol and IP3 (25). This IP3-mediated calcium release from intracellular compartments in a rapid and transient Ca2+ elevation that is most likely unrelated to the prolonged calcium elevation we observed using the PDGFR inhibitor.

ERK1/2 has a complex role in cellular differentiation. The prototypic, rapid MAPK signaling is mitogenic (26), yet prolonged ERK signaling is required for myeloid and monocytic differentiation (17, 18). In the current study, we did not observe a short-term ERK1/2 phosphorylation, and we cannot exclude the possibility the late increase in ERK phosphorylation is due to reduced potency of AG1296 after 24 to 48 h in cell culture media. We have previously shown that RA and D3 cause long-term ERK phosphorylation, and that the MAP/ERK kinase (MEK)/ERK inhibitor PD98059 blocks RA- and D3-induced differentiation of myeloblastic leukemia cells (18).

Although some differentiation markers are enhanced when AG1296 is administered along with RA or D3, we believe that there is a negative net impact of the presence of a PDGFR inhibitor during RA chemotherapy of APL patients, first, because the ultimate goal of RA treatment in APL patient is to obtain terminal differentiation and growth arrest. However, our data show that AG1296 inhibited G0 arrest. Second, we show that AG1296 increases cell adhesion. Increased adhesion could, however, aggravate retinoic acid syndrome (RAS), a complication of differentiation induction therapy. During RAS, RA causes adhesion of the leukemic cells to endothelial cells. This results in apoptosis of endothelial cells and leukocyte infiltration characteristic of the cardiopulmonary distress in RAS (27).

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References

9. Dubois-Dalcq M, Murray K. Why are growth factors used in this study.


1 M. Shen and A. Yen, unpublished data.


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