Regulation of progranulin expression in myeloid cells

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Submitted 24 August 2005; accepted in final form 21 July 2006

Ong, Colin H. P., Zhiheng He, Leonid Kriazhev, Xiaochuan Shan, Roger G. E. Palfree, and Andrew Bateman. Regulation of progranulin expression in myeloid cells. Am J Physiol Regul Integr Comp Physiol 291: R1602–R1612, 2006. First published July 27, 2006; doi:10.1152/ajpregu.00616.2005.—Progranulin (pgrn; granulin-epithelin precursor, PC-cell-derived growth factor, or acrogranin) is a multifunctional secreted glycoprotein implicated in tumorogenesis, development, inflammation, and repair. It is highly expressed in macrophage and monocyte-derived dendritic cells. Here we investigate its regulation in myeloid cells. All-trans retinoic acid (ATRA) increased pgrn mRNA levels in myelomonocytic cells (CD34+ progenitors; monoblastic U-937; monocytic THP-1; progranulocytic HL-60; macrophage RAW 264.7) but not in nonmyeloid cells tested. Interleukin-4 impaired basal expression of pgrn in U-937. Differentiation agents DMSO, and, in U-937 only, phorbol ester [phorbol 12-myristate,13-acetate (PMA)] elevated pgrn mRNA expression late in differentiation, suggestive of roles for pgrn in more mature terminally differentiated granulocyte/monocytes rather than during growth or differentiation. The response of pgrn mRNA to ATRA differs in U-937 and HL-60 lineages. In U-937, ATRA and chemical differentiation agents greatly increased pgrn mRNA stability, whereas, in HL-60, ATRA accelerated pgrn mRNA turnover. The initial upregulation of pgrn mRNA after stimulation with ATRA was independent of de novo protein synthesis in U-937 but not HL-60. Chemical blockade of nuclear factor-κB (NF-κB) activation impaired ATRA-stimulated pgrn mRNA expression in HL-60 but not U-937, whereas in U-937 it blocked PMA-induced pgrn mRNA expression, suggestive of cell-specific roles for NF-κB in determining pgrn mRNA levels. We propose that: 1) ATRA regulates pgrn mRNA levels in myelomonocytic cells; 2) ATRA acts in a cell-specific manner involving the differential control of mRNA stability and differential requirement for NF-κB signaling; and 3) elevated pgrn mRNA expression is characteristic of more mature cells and does not stimulate differentiation.

granulin; stem cell factor; colony-stimulating factor

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MATERIALS AND METHODS

Materials. Phorbol 12-myristate,13-acetate (PMA), protein kinase C (PKC) inhibitor, RO-31–8425, genistein, pertussis toxin, and nuclear factor-κB (NF-κB) activation inhibitor 6-amino-4-(4-phenoxynaphthalenyl)quinazoline (6-APQ) were obtained from Calbiochem (San Diego, CA). DMSO was supplied by ICN Biomedicals (Costa Mesa, CA). All-trans retinoic acid (ATRA), cycloheximide (CHX), and actinomycin D were purchased from Sigma (St. Louis, MO). Cytokines were obtained from Genetics Institute (Cambridge, MA) except interferon (IFN)-α (Schering Canada, Pointe Claire, Quebec, Canada), IL-4 (Biosource International, Camarillo, CA), and granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF; Leinco Technologies, St. Louis, MO). Recombinant human erythropoietin (EPO) was a gift from Dr. F. Congotte (Department of Medicine, Royal Victoria Hospital, Montreal, Quebec, Canada).

Cell culture. Cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and were maintained in the supplier’s recommended medium containing 10% FBS (Biomedia Canada, Drummondville, Quebec, Canada). The human bone marrow CD34+ hematopoietic progenitor cells were obtained from Cambrex (Walkerville, MA). Human dermal fibroblasts, TF-1, and HL-60R cells were gifts from Dr. A. Philip (Department of Surgery, Montreal General Hospital, Montreal, Quebec, Canada) and Dr. F. Congotte and Dr. S. Collins (Fred Hutchinson Cancer Research Center, Seattle, WA), respectively.

Cell lines of hematopoietic origin were seeded at a density of 10⁶ cells/ml. In the initial screen of pgrn mRNA regulation in U-937 cells, concentrations of the cytokines used were as follows: IFN-α (1,000 U/ml), IL-1α (10 U/ml), IL-2 (2.5 U/ml), IL-11 (1,000 ng/ml), stem cell factor (SCF; 10 U/ml), macrophage colony-stimulating factor (100 ng/ml), G-CSF (50 ng/ml), IL-3 (100 U/ml), IL-6 (100 U/ml), GM-CSF (1,000 U/ml), IFN-γ (100 U/ml), IL-4 (100 U/ml), and IL-5 (50 ng/ml). EPO was used at a final concentration of 5 U/ml. ATRA was used at 1 μM. CHX was used at 10 μg/ml. DMSO was used at a concentration of 1.25% (vol/vol). PMA was used at 100 nM. For chemical inhibitors, cells were pretreated with either RO-31–8425, 6-APQ, or CHX for 30 min before the addition of any differentiation stimulus. Actinomycin D was used at a concentration of 2 μg/ml.

Human hematopoietic colony-forming cell assay. Human bone marrow CD34+ hematopoietic progenitor cells were seeded in methycellulose-based media (MethoCult, StemCell Technologies, Vancouver, British Columbia, Canada) containing h (human) SCF, hGM-CSF, hIL-3, hIL-6, hG-CSF, and hEPO at a density of 700 cells/ml. ATRA was added to the suspension to final concentrations of either 10−7 or 10−6 M. The suspensions were then plated in 35-mm dishes and maintained at 37°C, 5% CO₂ for 14 days. Colonies were then observed through an inverted microscope and enumerated.

Real-time RT-PCR assay on human bone marrow CD34+ hematopoietic progenitor cells. Individual colony-forming unit granulocyte-macrophage (CFU-GM) colonies were picked and pooled (~30 colonies) for total RNA extraction using Tri-Zol Reagent (GIBCO-BRL/Life Technologies). The RNA was treated with RNase-free DNase (Promega, Madison, WI) and reverse transcribed using random hexamers (Promega). For quantitative real-time PCR, the primer pairs used for the pgrn transcript were 5′-GGACAGTACTGGAAGACTCTG-3′ (forward primer) and 5′-GGATGGACGCTGTGTAATGG-3′ (reverse primer), whereas the primers for β-actin were 5′-GAATGTTGACGTTGACATCC-3′ (forward primer) and 5′-CCGATCACGACGAGCTT-3′ (reverse primer); GIBCO-BRL/Life Technologies. Amplification reaction mixtures were prepared according to the manufacturer’s instructions using a LightCycler FastStart DNA Master SYBR Green I kit (Roche Applied Science, Penzberg, Germany) with a final primer concentration of 0.5 μM for each reaction. The amplifications, in duplicates, were performed in the LightCycler (Roche Applied Science) following the conditions: hot start step (denaturation) at 95°C for 10 min followed by 45 cycles of 95°C for 10 s, 66°C for 10 s, and 72°C for 10 s. The expression of GRN was normalized to that of β-actin. Gene expression was expressed as N-fold differences where: N = 2ΔCp for GRN − ΔCp for β-actin. ΔCp is the difference between the crossing point values for ATRA-treated and untreated CD34+ hematopoietic progenitor cells.

Isolation of total RNA and Northern blotting. Total RNA was isolated from 10⁶ cells using Tri-Zol Reagent. RNA samples, 10 or 15 μg, were denatured with glyoxal at 50°C for 1 h and subjected to electrophoresis using a 1% agarose gel in 10 mM Na₂HPO₄, pH 7. Northern blot analysis was then performed as described previously (4). Densitometry was performed using Scion Image software and normalized to the 28S ribosomal RNA bands. A relative density of one was assigned to the control sample. Values were presented as mean values ± SE. Statistical analyses were performed using the Student-Newman-Keuls test (Graphpad Instat software).

Isolation of recombinant human pgrn. Recombinant human pgrn was made by transient transfection of pgrn/pCDNA3 in COS-7 cells and purified using a C4 reversed-phase HPLC column as described previously (23). The biological activity of pgrn was monitored by its ability to promote the growth of SW-13 cells as described previously (23).

Cell differentiation and cytofluorometric analysis. Cellular differentiation status was assessed by morphologically scoring 100 cells at each time point using the Wright-Giemsa staining method (34). The induction of CD11b was determined using 10⁶ cells resuspended in a total volume of 50 μl with PBS/0.1% NaN₃/1% FBS. After being blocked with normal human AB serum (Sigma) and normal mouse IgG (Caltag Laboratories, Burlingame, CA), the cell suspension was incubated with 20 μl of phycoerythrin-conjugated anti-CD11b (BD Biosciences, Mississauga, Ontario, Canada) at 4°C for 45 min in the dark. The cells were then washed twice and resuspended in 1% paraformaldehyde in PBS/0.1% NaN₃ and analyzed for CD11b by cytofluorometry (Clinical Research Institute of Montreal).

Western blot analysis for pgrn. Cells (10⁶ cells/ml in serum- and phenol red-free RPMI-1640 medium) were exposed to either 1 μM ATRA, 100 nM PMA, or 1.25% (vol/vol) DMSO for 48 h. Conditioned medium (CM) and cell pellets were collected by centrifugation at 170 g at room temperature for 5 min. Samples were extracted with an equal volume of 2× (CM) or 10 ml 1× (cell pellet) extraction medium [1X: 1 M HCl, 0.17 M NaCl, 1% (vol/vol) trifluoroacetic acid, and 4.5% (vol/vol) formic acid]. The CM extraction was mixed well, and the cell pellet was subjected to sonication using an ultrasonic homogenizer (Cole Palmer Instruments, Chicago, IL). The mixtures were then centrifuged at 2,000 g at 4°C for 25 min to remove debris. Extracts were desalted and concentrated using a Waters C₁₈ Sep-Pak cartridge (Milford, MA) as described previously (3) and lyophilized. (The efficient adsorption of pgrn to C₁₈ cartridges was confirmed using recombinant human pgrn.) The samples were resuspended in milliQ water and a volume equivalent to 3.0 × 10⁶ cells was subjected to SDS-PAGE (12%). After protein transfer to nitrocellulose membranes (Hybond-C extra; Amersham Pharmacia Biotech, Baie d’Urfe, Quebec, Canada), the membranes were blocked with 5% nonfat milk and probed with a rabbit polyclonal anti-human pgrn antibody (1:10,000, prepared by Dr. G. Sadvakassova, Royal Victoria Hospital) followed by donkey anti-rabbit antibody conjugated to horseradish peroxidase (1:5,000). Antigen was visualized by chemiluminescence using luminol as substrate (Amersham Pharmacia Biotech). Identical duplicate blots were probed with preimmune serum.

RESULTS

Regulation of pgrn mRNA levels by retinoic acid. The monoblastoid U-937 cell line can be stimulated to differentiate toward more mature monocyte-like cells (22, 42) or adherent macrophage-like cells (40). When we exposed U-937 cells to a panel of cytokines and other physiological mediators that regulate myeloid maturation or function, ATRA stimulated a
strong induction of pgrn transcript levels, whereas IL-4 decreased basal pgrn levels (Fig. 1A). ATRA stimulated pgrn mRNA expression by several cells in the granulocyte/monocyte lineage [CFU-GM derived from human bone marrow CD34+/H11001 hematopoietic progenitor cells (Fig. 1B) and human acute monocytic leukemic cell line THP-1 (Fig. 2A and supplemental Fig. 1A) and NB4 (preliminary data, not shown)]. A small but statistically significant pgrn mRNA elevation occurred in ATRA-stimulated RAW 264.7, a well-differentiated macrophage-like cell line (Fig. 2A and supplemental Fig. 1A). There was no increase in pgrn mRNA levels in HL-60R cells (Fig. 2A and supplemental Fig. 1A), which are HL-60 cells with a mutated retinoic acid receptor-α (RAR-α), implying a requirement for RAR-α in the stimulation of pgrn expression (49).

The induction of pgrn mRNA by ATRA was rapid, with the initial increase observed at 6 h or less for U-937, HL-60, and THP-1 cells (Fig. 2A). ATRA had no effect on pgrn mRNA levels in the nonmyelomonocytic cell lines THP-1 (proerythroid), Jurkat (lymphoid), SW-13 (epithelial), primary fibroblasts, or U373-MG neuroblasts (not shown).

ATRA-stimulated maturation of U-937 and HL-60 cells was assessed by the expression of the cell surface differentiation marker CD11b (Fig. 2Bi) or morphology (Fig. 2, Bi and Biii). Treatment of U-937 or HL-60 cells with pgrn for 4 days with or without ATRA had no effect on CD11b expression (Fig. 2Bi), morphology, or cell number (not shown).

1 The online version of this article contains supplemental data.
In HL-60, but not U-937, the DMSO upregulation of pgrn mRNA requires serum, since DMSO inhibited rather than stimulated pgrn expression in serum-free HL-60 cultures (Fig. 4A). The PKC inhibitor RO-31–8425 inhibited the DMSO-serum costimulation of pgrn expression (Fig. 4B), whereas genistein, a protein tyrosine kinase inhibitor, and pertussis toxin, an inhibitor of Gi protein signaling, had no effect (not shown).

To investigate whether expression of pgrn mRNA occurs whenever hematopoietic cells differentiate or is more specific to myelomonocytic cells, we used the cytokine EPO to stimulate differentiation of TF-1 proerythroid cells. No increase in pgrn mRNA levels was seen in TF-1 cells stimulated by EPO for 5 days (Fig. 5). Erythroid differentiation was confirmed by benzidine staining.

ATRA-mediated upregulation of pgrn mRNA levels requires de novo protein synthesis in HL-60 but not in U-937 cells. The GRN promoter lacks canonical retinoic acid response elements (RAREs). We therefore postulated that ATRA stimulation of pgrn mRNA expression would not be mediated through direct actions on the GRN promoter but would be a secondary action of ATRA requiring intermediate gene induction and de novo protein synthesis. This was investigated using CHX, a protein synthesis inhibitor (32). In ATRA-stimulated HL-60 cells, CHX suppressed the upregulation of the pgrn transcript, whereas CHX on its own had no effects (Fig. 6B). Therefore, the action of ATRA on pgrn mRNA levels in HL-60 is, as we proposed, indirect. In contrast, CHX did not inhibit the initial increase of pgrn mRNA levels in U-937 in response to ATRA (Fig. 6A), implying no requirement for a secondary intermediate in U-937.

The stability of pgrn mRNA is differentially regulated during maturation along monocytic and neutrophilic pathways. To investigate the stability of pgrn transcript in differentiated myelogenous cells, we stimulated differentiation for 48 h with ATRA, DMSO, or PMA, blocked further transcription with actinomycin D, and then determined the rate of mRNA turnover by performing a Northern blot time course. There was a considerable enhancement of pgrn mRNA stability in U-937 cells when exposed to ATRA, DMSO, or PMA, implying that the rate of mRNA turnover decreases when U-937 cells are stimulated to differentiate along the monocytic lineage (Fig. 7A). In contrast, in HL-60 cells, the degradation of pgrn transcript became more rapid upon stimulation with ATRA or DMSO (Fig. 7B).

Chemical inhibition of the NF-κB pathway and GRN expression. Interaction of NF-κB and retinoid signaling is known to regulate gene expression during myelopoiesis (64). A potent
inhibitor of NF-κB activation, 6-APQ (60), inhibited pgrn mRNA expression stimulated by ATRA in HL-60 cells (Fig. 8D) and by PMA in U-937 cells (Fig. 8C). 6-APQ did not inhibit the pgrn mRNA response to ATRA in U-937 cells (Fig. 8A), supporting distinct mechanisms of action of ATRA on pgrn transcript expression in HL-60 vs. U-937 cells. 6-APQ did not inhibit the DMSO-induced increase in pgrn mRNA in either cell line (Fig. 8, B and E). Differentiation of U-937 cells to adherent cells by PMA was inhibited by the presence of 6-APQ (PMA alone: 98.7% adherent, 1.3% nonadherent; PMA with 5 μM 6-APQ: 18.8% adherent, 81.2% nonadherent; PMA with 10 μM 6-APQ: 18.9% adherent, 81.1% nonadherent). The specificity of the response to 6-APQ eliminates the possibility that the results are because of toxic effects of the drug.

IL-4 inhibits basal and ATRA-stimulated expression of pgrn mRNA in U-937 cells. The initial screen of putative regulators of pgrn expression identified IL-4 as an inhibitor of pgrn expression (Fig. 1A). IL-4 decreased pgrn mRNA levels of U-937 cells at concentrations of 2 and 20 ng/ml, whereas, at 60 ng/ml, IL-4 had no inhibitory effect (Fig. 9A). The pgrn mRNA levels were suppressed after 12 h by 20 ng/ml IL-4 (Fig. 9B). IL-4 had no effect on pgrn transcript levels in HL-60 cells (Fig. 9A). G-CSF and GM-CSF, which, like IL-4, also employ JAK-STAT signaling pathways and appeared to lower pgrn mRNA in the initial screen (Fig. 1A), had only small and nonstatistical effects on pgrn mRNA levels in U-937 and HL-60 cells (data not shown). To investigate the interactions between the negative regulatory signals from IL-4 and stimulation by ATRA, DMSO, and PMA, we coincubated U-937 cells with IL-4 and each of the differentiation agents. IL-4 significantly inhibited pgrn mRNA stimulation by each agent (Fig. 9C).

DISCUSSION

The major findings of this study are summarized in Fig. 10. The pgrn is regulated by retinoic acid in granulocyte and monocyte cell lineages that retain the ability to differentiate (primary bone marrow CD34+ progenitors, U-937, THP-1, and HL-60 cells), whereas the well-differentiated RAW 264.7 macrophage line displayed only minor ATRA stimulation. The pgrn expression in representative nonmyeloid cells was insensitive to ATRA.

The mechanisms by which ATRA stimulates pgrn mRNA expression in monoblastoid U-937 and promyelocytic HL-60 cells are distinct. ATRA regulates early pgrn expression in HL-60 cells indirectly, requiring de novo protein synthesis, as expected for a gene with no canonical RARE, but in U-937 protein synthesis was not required. Presumably in U-937 the
ATRA-receptor complex acts directly at the GRN promoter but through a nonclassical mechanism as has been reported, for example, for the urokinase plasminogen activator gene (59). Similarly, we can distinguish two mechanisms of ATRA-stimulated pgrn expression using the NF-κB inhibitor 6-APQ, which blocks pgrn regulation by ATRA in HL-60 but not U-937 cells. NF-κB and retinoic acid signaling pathways have recently been shown to interact in other systems, including NB4 promyelocytic cells (64) and keratinocytes (13). Other nuclear hormones, such as estrogen, also stimulate pgrn expression in, for example, breast cancer cells (35, 36) and neonatal hypothalamus (57), and it will be of interest to determine whether different regulatory pathways are employed by these hormones in their different target cells.

Although ATRA is anti-inflammatory for mature macrophages and is used to treat acne and psoriasis, it promotes the maturation of myeloid precursor cells toward monocyte and granulocyte-like cells (7, 11, 22, 42) and induces remission in acute promyelocytic leukemia. Because pgrn mRNA increased in parallel with differentiation after ATRA stimulation, it was pertinent to investigate whether pgrn might regulate differentiation or be expressed as a consequence of differentiation. We first tested its ability to regulate maturation and then examined the time course of pgrn expression when differentiation was stimulated independently of ATRA using pharmacological stimulants. The pgrn had no obvious effect on differentiation either with or without ATRA. The absence of increased pgrn expression in the PMA-induced differentiation of HL-60 cells suggests that their maturation, at least as far as promonocytes, does not require increased pgrn expression. Furthermore, the upregulation of pgrn transcript levels with chemical differentiation agents occurred only after differentiation had begun (HL-60 with DMSO) or was complete (U-937 with PMA). The inability of pgrn to stimulate differentiation, and the temporal displacement between the onset of differentiation and pgrn expression in PMA- and DMSO-stimulated cells, argue against

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**Fig. 4.** A: serum is required for DMSO-mediated elevation of pgrn transcript levels in HL-60 cells. Northern blots for pgrn were performed on RNA from HL-60 cells exposed to either ATRA, DMSO, or PMA for 48 h in the presence (+FBS) or absence (−FBS) of serum (n = 3). P values shown are for the experiments vs. the respective controls. B: upregulation of pgrn mRNA mediated by DMSO + FBS is inhibited by RO-31–8425, a PKC inhibitor, cells exposed to the inhibitor alone are labeled 2 (2 μM) and 10 (10 μM) (n = 3). *P < 0.05, **P < 0.01, and ***P < 0.001. D, DMSO. C: secreted pgrn protein levels in serum-free CM and intracellular pgrn levels (from cell pellet fraction) detected in U-937 and HL-60 cells grown in serum-containing medium. U-937 cells (i and iii) and HL-60 cells (ii and iv) were exposed to either ATRA, DMSO, or PMA for 48 h, and protein was extracted and subjected to anti-pgrn Western blot analysis. Untreated cells are labeled as control. The positions of pgrn and the fragment (Frag) of pgrn are marked by the arrows on the left, whereas the molecular weight markers are represented by the arrows on the right of the blots. Rec pgrn, recombinant pgrn.
a role for progranulin in differentiation but are consistent with the hypothesis that progranulin is a secretory product of mature macrophage and granulocytes both as an inflammatory modulator (67) and growth factor (25). The regulation of hematopoiesis is, however, multifactorial, involving the synergistic interactions of stromal or autocrine growth factors, cytokines, and chemokines, so we cannot rule out the possibility that progranulin regulates maturation in concert with other factors. The progranulin promotes cell survival (28), so it will be of interest to investigate whether it also impairs apoptosis in myeloid cells.

The progranulin was secreted as a single high-molecular-weight protein except in ATRA-stimulated HL-60 cells, where an additional 26-kDa progranulin-immunoreactive band was detected, which we assume is a fragment of progranulin. This could be because of differences in secretion of elastase or SLPI, which awaits further investigation.

PMA acts on myeloid differentiation by activating the PKC-βII isoform of PKC (45, 53). PMA-stimulated progranulin expression is blunted in the PKC-βII-deficient TUR strain compared with U-937. The lower-level expression of progranulin mRNA that persists in TUR is probably maintained via activation of other PKC isoforms.

Fig. 5. TF-1 cells that mature along the erythroid lineage do not display an increased expression of the progranulin transcript. Northern blots were performed for progranulin from TF-1 cells induced to differentiate with erythropoietin (EPO). The experiment was performed three times. ns, Not significant.

Fig. 6. Requirement for de novo protein synthesis in ATRA-stimulated upregulation of progranulin mRNA in U-937 and HL-60 cells. U-937 (A) and HL-60 (B) were treated with either 1 μM ATRA alone, 10 μg/ml CHX alone, a combination of both 1 μM ATRA and 10 μg/ml CHX, or subjected to no treatment at all (vehicle control) followed by Northern blot analysis of progranulin mRNA. All of the experiments were carried out at least 3 times. *P < 0.05, **P < 0.01, and ***P < 0.001.

Fig. 7. The progranulin transcript stability. U-937 (A) and HL-60 (B) cells were stimulated with differentiation agents, ATRA, DMSO, or PMA for 48 h, and then actinomycin D (2 μg/ml) was added. RNA isolated at various times after the addition of actinomycin D was subjected to Northern blot analysis. The progranulin transcript levels are expressed as percentages of levels at time 0. Each experiment shown was performed 3 times.
isoforms. 6-APQ blocks PMA-stimulated pgrn expression in U-937 cells. Activation of PKC by PMA is known to induce DNA binding by the NF-κB subunits p65 and p50 in U-937 and monocytic cells (30, 45, 53), and blockade of the NF-κB pathway in U-937 prevents PMA-stimulated differentiation (14, 19). 6-APQ inhibits PMA-induced differentiation, and the inhibition of pgrn mRNA expression by 6-APQ in PMA-treated U-937 cells may therefore result from blocked differentiation.

Posttranscriptional regulation of mRNA levels is important in the physiological control of cytokine expression (5, 54) and, as we show here, in the expression of pgrn. ATRA or chemical differentiation agents induced higher stability of the pgrn transcript in U-937 but reduced its stability in HL-60, providing further evidence for differential mechanisms in the control of pgrn mRNA expression. The molecular basis for differential regulation of pgrn mRNA stability is at present unknown, and analysis of the 5'- and 3'-untranslated regions and coding region of pgrn mRNA using UTRscan (www.ba.itb.cnr.it/BIG/UTRHome; see Ref. 46) revealed no obvious mRNA stability elements. The differential stability of pgrn mRNA may have physiological consequences. An unstable pgrn message will be transient after the cessation of stimuli, as was observed in neutrophils after microbial stimulation (55), whereas the steady-state abundance of pgrn mRNA in human macrophages (9) and dendritic cells (21) may result in part from longevity of the pgrn transcript. Intriguingly, the stabilization of pgrn mRNA is paralleled at the protein level where intact pgrn is stabilized by SLPI (41, 67). We propose that the differential turnover of the mRNA and the protein may both be critical in determining the overall physiological levels of pgrn. It will be important to determine whether stabilization of pgrn mRNA occurs in other systems, particularly in cancer, where a high

Fig. 8. The pgrn mRNA induction by PMA in U-937 and by ATRA in HL-60 cells is suppressed by 6-amino-4-(4-phenox-phenylethylamino)quinazoline (6-APQ), a nuclear factor-κB (NF-κB) activation inhibitor. U-937 or HL-60 cells were treated with either 5 or 10 μM of 6-APQ for 30 min before the addition of one of the differentiation agents ATRA, DMSO, or PMA. After 48 h, cells were harvested, RNA was extracted, and Northern analysis was performed. Results are shown for ATRA [U-937 (A) and HL-60 (D)], DMSO [U-937 (B) and HL-60 (E)], and PMA [U-937 (C)]. Cells exposed to the inhibitor alone are labeled as “5” (5 μM) and “10” (10 μM); n = 3 for U-937/PMA/6-APQ and HL-60/ATRA/6-APQ and n = 2 for all the other experiments. ***P < 0.001.

Fig. 9. Differential effects of IL-4 on the regulation of pgrn mRNA in U-937 and HL-60 cells. A: IL-4 dose dependence of pgrn transcript levels in U-937 and HL-60 cells (incubation time: 20 h). B: time course studies (with 20 ng/ml IL-4) on U-937 cells. C: competitive effects of IL-4 and ATRA, DMSO, or PMA on pgrn mRNA levels in U-937 cells. The cells were treated with IL-4 (20 ng/ml) with or without 1 μM ATRA, 1.25% (vol/vol) DMSO, or 100 nM PMA for 20 h, and Northern analysis was performed. All experiments were performed at least 3 times, with the exception of the dose-response curve for HL-60 stimulated with IL-4 (A) where n = 2. *P < 0.05, **P < 0.01, and ***P < 0.001.
level of pgrn is often associated with advanced disease (10, 18, 26, 33, 44, 51).

During myelopoiesis, the levels of gene expression are fine tuned through the integration of positive and negative signals. We identified IL-4 as an inhibitor of basal and ATRA-stimulated pgrn expression in U-937 but not in HL-60 cells, even though HL-60 expresses the IL-4 receptor (61). This further demonstrates cell specificity in the regulation of pgrn mRNA expression and is to our knowledge the first report of negative regulation of GRN expression by a physiological mediator.

In conclusion (Fig. 10), retinoic acid upregulates pgrn mRNA levels in myelomonocytic cells. The pgrn appears not to mediate differentiation but to be an outcome of differentiation. The stimulation of pgrn expression by ATRA and chemical differentiation agents shows marked differences between cells in terms of 1) a requirement for intermediate protein synthesis after ATRA stimulation, 2) sensitivity to inhibition by 6-APQ, 3) serum dependence of the response to DMSO, and 4) posttranscriptional regulation of mRNA stability. These results argue for great caution when generalizing results to other cells, even to cells of close lineage. The ability of physiological mediators such as ATRA and IL-4 to regulate pgrn mRNA in myelomonocytic cells, but through distinct, cell-specific mechanisms, demonstrates a complex regulatory repertoire for pgrn expression in the innate immune system that may be crucial for the appropriate coordination of the processes it affects, such as cell survival, proliferation, and migration at sites of infection and wound healing.

ACKNOWLEDGMENTS

We thank the Molecular Oncology Unit, Royal Victoria Hospital, Montreal, Quebec, Canada, for usage of the LightCycler machine and Dr. L. Fernando Congote for invaluable advice.

Present addresses: Z. He, Section on Vascular Cell Biology, Joslin Diabetes Center, Harvard Medical School, Harvard University, Boston, MA; L. Kriazhev, Genome Quebec, McGill University, Montreal, Quebec, Canada; and X. Shan, Clinical Cell and Vaccine Production Facility, University Of Pennsylvania, Philadelphia, PA.

GRANTS

This work was supported by Canadian Institutes of Health Research Grant MT11288. C. H. P. Ong was a recipient of the McGill University Health Centre Research Institute Studentship.
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