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Changes in Proteoglycan Core Protein (PCP) mRNA Levels During HL-60 Cell Differentiation

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Summary. The steady-state level of the PCP mRNA was investigated during HL-60 cell differentiation. When cells were induced to differentiate into granulocytes with 1.3% dimethylsulfoxide or 1 μM cis or trans retinoic acid, no significant changes in PCP mRNA levels were observed. In contrast, cells cultured in the presence of the phorbol ester TPA, which promotes the cells to be differentiated into monocytes/macrophages, were associated with a marked decrease in the steady-state concentration of PCP mRNA. When cells were simultaneously treated with a combination of TPA and staurosporine, a protein kinase C (PKC) inhibitor, the TPA-elicited decrease in PCP mRNA was partially prevented. These data suggest that PCP mRNA expression is not directly linked to HL-60 cell differentiation but appears to involve the participation of PKC.
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The HL-60 cells, isolated from a patient with acute promyelocytic leukemia, have been widely employed to investigate the process of myeloid differentiation (1-3). HL-60 cells can be induced to differentiate into granulocytes with dimethylsulfoxide (DMSO) or retinoic acid (RA) (4-10), and into monocytes/macrophages with the tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA) or 1,25-dihydroxyvitamin D_3 (Vit D_3) (7, 11-16). Proteoglycans are macromolecules composed of one or more sulfated glycosaminoglycan chains covalently linked to a core protein. In HL-60 cells, the glycosaminoglycan is predominately chondroitin sulfate (17). A number of studies analyzing [^{35}S]-sulfate incorporation into chondroitin sulfate in HL-60 cells have shown that treatment with

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TPA, RA, or DMSO resulted in a 20-30%, 30-50%, and 45% decrease, respectively, in sulfate incorporation (17-20). These data suggest a correlation between proteoglycan synthesis and differentiation of HL-60 cells into different phenotypes. Proteoglycan biosynthesis can be regulated at various levels, such as - modulating the expression of the gene for the core protein, the processing of the core protein in the endoplasmic reticulum, the addition of the glycosaminoglycan chains and their modification in the golgi, packaging and secretion of the mature proteoglycan. Since the report of Hasumi et al. (18) suggested that proteoglycan synthesis may be determined by the concentration of the core protein, we investigated whether the core protein mRNA expression is correlated with HL-60 cell differentiation, by utilizing the recently isolated and characterized cDNA encoding the HL-60 proteoglycan core protein (PCP) (21, 22). This communication reports on the changes in PCP mRNA steady state levels in HL-60 cells induced to differentiate by treatment with TPA, DMSO, RA, or Vit D_3.

MATERIALS AND METHODS

HL-60 cells, obtained from ATCC, were maintained in RPMI 1640 medium (with L-glutamine) supplemented with 10% fetal bovine serum, penicillin (100 units/ml) and streptomycin (100 μg/ml) at 37°C, in a 5% CO_2 humidified incubator. Cells were seeded at an initial density of $3x10^5$ cells/ml for treatment with 16 nM TPA, 10 nM staurosporine, or TPA and staurosporine combined, and $1.5x10^5$ cells/ml for treatment with 1.3% DMSO, 1 μM RA, or 1 μM Vit D_3. Vit D_3 was the generous gift of Dr. M. Uskokovic (Hoffman-LaRoche, Nutley, N.J.).

Total RNA was isolated from control and treated cell pellets based on the method of Chomczynski and Sacchi (23). Fifteen-μg RNA samples were denatured in glyoxal as described by Sambrook and Maniatis (24), electrophoresed through a 1% agarose gel, directly transferred to a nylon membrane, and UV cross-linked. The HL-60 PCP cDNA was graciously donated by Dr. R.L. Stevens (21, 22). The 18S rRNA cDNA used to normalize the data was a generous gift from Dr. I. Wool (25) while the c-*myc* DNA, 3rd exon, was purchased from Oncor. The nylon filters were prehybridized, hybridized with labeled cDNA, and analyzed according to the procedure of Chang and Wu (26).

RESULTS AND DISCUSSION

HL-60 cells were treated with 16 nM TPA for 6, 12, 18, and 24 hours and the steady-state level of PCP mRNA was determined by

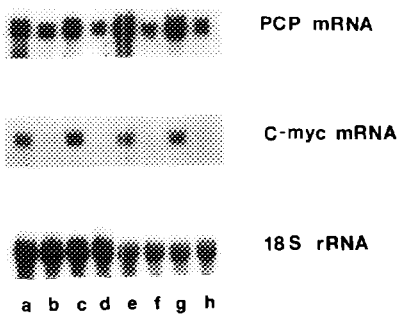

Figure 1. Northern analysis of PCP expression in TPA treated HL-60 cells. HL-60 cells were treated with 16 nM TPA for 6, 12, 18, and 24 hours. Fifteen-μg samples of total RNA isolated from the cells were electrophoresed through a 1% agarose gel, transferred to a nylon membrane, and hybridized with PCP and c-*myc* probes. 18S RNA was used to normalize the data. Lanes a, c, e, and g were the controls while b, d, f, and h were the corresponding TPA treated samples at 6, 12, 18, and 24 hours, respectively.

Northern analysis. A representative autoradiogram is shown in Figure 1. PCP mRNA levels decreased 28.2%, 53.5%, 70.2% and 60.5% after treatment with TPA for 6, 12, 18, and 24 hours, respectively. As confirmation of cell differentiation, mRNA levels of c-*myc*, which is known to be down-regulated during HL-60 differentiation (27), were determined and found to decrease by 85.5%, 97.2%, 95.7%, and 92.1% after treatment with TPA for 6, 12, 18, and 24 hours, respectively (Figure 2). Northern analysis of the steady-state level of PCP mRNA was also performed after treatment of HL-60 cells with various differentiating agents (Figure 3). Densitometry and normalization of the data showed that there were only negligible changes in PCP mRNA levels. However, c-*myc* mRNA levels decreased 88.3%, 82.6%, 97.5% and 90.8% when cells were treated with DMSO, Vit D_3, cis-RA, and trans-RA, respectively, showing that these agents elicited a typical differentiated cell response. These data suggest that proteoglycan synthesis is not associated with HL-60 cell differentiation. Our findings are in general agreement with the recent report by Stellrecht *et.al.* (28) in which they showed that whereas HL-60 cells cultured in the presence of 160 nM TPA

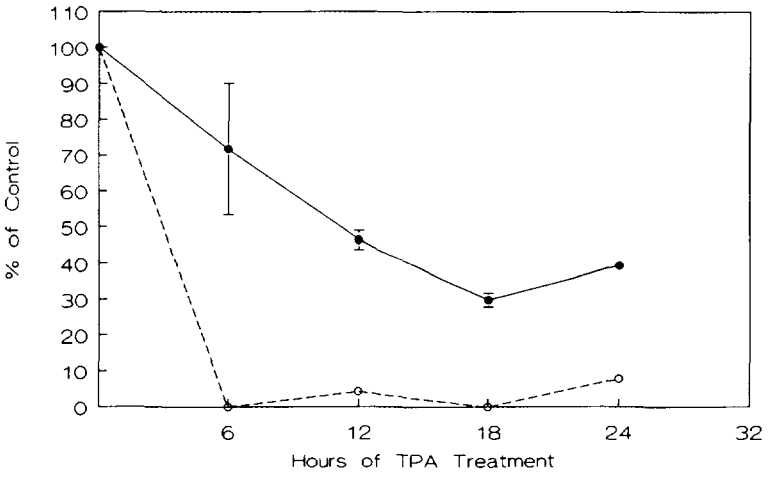

Figure 2. Time course analysis of PCP expression in TPA treated HL-60 cells. Autoradiograms from Northern analysis of HL-60 cells treated with 16 nM TPA for 6, 12, 18, and 24 hours were quantitated by densitometry and the control values arbitrarily adjusted to 100 %. The closed circles represent PCP mRNA while the open circles represent c-*myc* mRNA. Results represent the average of two independent measurements ± standard error (SE).

were accompanied by a 20-fold decrease in PCP mRNA content, treatment with RA or DMSO did not elicit any PCP mRNA level change.

Of the different inducing agents tested, only TPA caused a marked change in the steady-state level of PCP mRNA. TPA is known

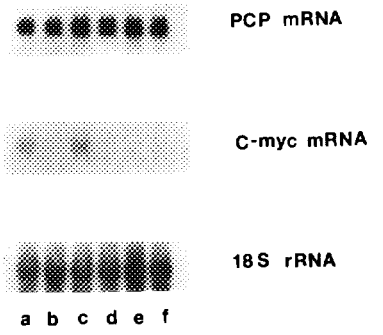

Figure 3. Northern analysis of PCP expression in differentiated HL-60 cells. HL-60 cells were treated with 1.3% DMSO, 1 μM Vit D_3, 1 μM RA (cis), or 1 μM RA (trans), for 6 days. Fifteen-μg samples of total RNA isolated from the cells were electrophoresed through a 1% agarose gel, transferred to a nylon membrane, and hybridized with PCP and c-*myc* probes. 18S RNA was used to normalize the data. Lanes a and c, controls; lane b, DMSO; lane d, Vit D_3; lane e, RA (cis); lane f, RA (trans).

to directly activate protein kinase C (PKC), whose activation and subsequent participation in signal transduction has been implicated in such cellular processes as growth and differentiation (29-32). PKC activity in HL-60 cells has been shown to increase after treatment with DMSO, RA, and Vit D_3 and to decrease after TPA treatment (33). In order to determine whether PKC has a possible role in PCP gene regulation, HL-60 cells were treated with the PKC inhibitor, staurosporine, alone and in combination with TPA. It was found that the steady-state levels of PCP mRNA decreased considerably after treatment with 16 nM TPA, and increased to a small extent with 10 nM staurosporine; combination of both reagents resulted in a 61% decrease (Table 1). The finding that staurosporine counteracted the suppressive effect of TPA thus strongly suggests the participation of PKC in PCP regulation. The inability of staurosporine to completely abolish the inhibitory effect of TPA on the PCP mRNA may be due to its ability to inhibit other protein kinases, e.g., tyrosine and cyclic AMP-dependent

TABLE 1. NORTHERN ANALYSIS OF STAUROSPORINE TREATMENT

Treatment		PCP mRNA		C-*myc* mRNA	
Agent	Time	Percent of Control	Percent of Change	Percent of Control	Percent of Change
16ηM TPA	1 day	7.5±2.2	92.5 ↓	2.7	97.3 ↓
10ηM Staurosporine	1 day	102.3±7.6	2.3 ↑	128.2	28.2 ↑
10ηM Staurosporine Plus 16ηM TPA	1 day	39.0±13.2	61.0 ↓	21.2	78.8 ↓

Analysis of PCP expression in HL-60 cells treated with TPA, staurosporine, or TPA and staurosporine combined. Fifteen-μg samples of total RNA isolated from control and treated cells were electrophoresed through a 1% agarose gel, transferred to a nylon membrane, and hybridized with PCP and c-*myc* probes. 18S RNA was used to access uniformity of RNA loading and to normalize the data. Autoradiograms were quantitated by densitometry and the control values arbitrarily adjusted to 100%. Results are the average of the following number of independent measurements ± standard error (SE): for PCP: TPA, TPA plus staurosporine, 3; staurosporine, 5; c-*myc* was done only once.

protein kinases (34-36), in addition to PKC. An alternative explanation is that in our experiments, both agents were added simultaneously to the HL-60 cells, thereby allowing staurosporine to be only partially effective in inhibiting PKC. Preincubation of the cells with staurosporine before the addition of TPA as well as the use of more specific inhibitors of PKC will be necessary to further elucidate the participation of PKC in regulating PCP gene expression. Contrary to expectation, treatment with a different monocyte-macrophage inducing agent, Vit D_3, did not result in a change in PCP mRNA (Figure 3). The differential effects of TPA and Vit D_3 on PCP mRNA changes may be explained by the subtle changes brought about by PKC isozyme gene expression. Specifically, studies on the effect of TPA on mRNA levels of the PKC-α and PKC-β isozymes have shown that it causes a decrease in the PKC-α and an increase in the PKC-β isozymes (37), whereas both isozyme mRNA levels were increased with Vit D_3 treatment (38,39). Studies by Edashige *et.al.* (40), involving the use of TPA and PKC inhibitors, suggest that the up-regulation of PKC-α is more closely related to the TPA-induced HL-60 differentiation. Taken together, our findings strongly suggest a role for PKC-α in the regulation of PCP gene expression. Studies are currently underway to examine this possibility.

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