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LYN, A SRC-LIKE TYROSINE-SPECIFIC PROTEIN KINASE, IS EXPRESSED IN HL60 CELLS INDUCED TO MONOCYTE-LIKE OR GRANULOCYTE-LIKE CELLS

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Summary: During the in vitro differentiation of HL60 cells tyrosine-specific kinases are activated. The expression of lyn, a src-related tyrosine kinase, was studied by analysis of the steady-state levels of its transcript during the cell differentiation process induced by retinoic acid, phorbol 12-myristate 13-acetate and 1,25-dihydroxyvitamin D_3. In contrast to an earlier report we observe only a small induction of the lyn-RNA levels compared to uninduced control cells. In unstimulated HL60 cells, the level for the lyn-transcript was comparatively high. A second, minor human lyn-transcript with an estimated size of 3.7kb which has not been previously described, was identified.

Tyrosine-specific protein kinase activity was originally demonstrated in viral gene products (1). Structural analysis of the cellular homologues of these viral oncogenes revealed two distinct sets. members of the first group are transmembrane proteins and are in any cases receptors for growth factors whereas in the second class src-like kinases are grouped together. These src-related proteins do not carry extracellular or transmembrane sequences, but they are closely associated with the internal portion of the cell membrane by myristoylation at their aminotermini (1). This suggests a function in the signal transduction pathway that is broadly responsible for regulating cell proliferation and differentiation.

In the hematopoietic system both cellular events are controlled through a network of cytokines and their receptors (2,3) which, with a single exception, do not represent tyrosine-specific kinases (4). Nevertheless, it has been shown that activation of these cytokine receptors resulted in a general increase of protein tyrosine phosphorylation in the cells (5,6). Several studies now suggest that these src-like proteins, 8 are known to date, are responsible in lymphatic and myeloid cell lineages for the observed induction of tyrosine phosphorylation. Protein tyrosine phosphatases, for example the CD 45

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Abbreviations: PMA, phorbol 12-myristate 13-acetate; MPO, myeloperoxidase; 1,25 $[OH]_2$ D_3, 1,25 dihydroxyvitamin D_3.

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antigen cluster, may act as the counter regulator to tyrosine kinases (7). The up-regulation of CD45 mRNA (8) and the stimulation of protein tyrosine phosphorylation activity (9) during induced differentiation of HL60 cells was reported. This encouraged us to study in these cells the expression of lyn, a protein tyrosine kinase of the src-family known to be expressed in most types of hematopoietic cells, including macrophages and monocytes (10).

MATERIALS AND METHODS

Cell culture: HL60 cells were cultivated in enriched Mc Coy's medium (Gibco BRL) as described (11). Cell viability was estimated in all experiments by trypan-blue. 12-16hrs before addition of the inducer substances, cell cultures with a density of about $1x10^6$ cells/ml were harvested and resuspended in fresh medium to a density of $0.8x10^6$ cells/ml. After three days of culture, the medium of the stimulated cells was completely exchanged and fresh inducers were added.

Total RNA extraction and Northern blot analysis: Both procedures were done as described recently (11) except that hybridisation with the human lyn and human ß-actin probes were done at 42° C and that washing of the blots was done in 2xSSC, 0.1% SDS at RT for 30min and at 65°C in 0,2xSSC, 0.1% SDS for 30min. To exclude an unspecific binding of [α-^{32}P] radiolabelled lyn-cDNA, the blots were rewashed 2x 30min in 0.1x SSC, 0.1% SDS at 65°C. The lyn-probe was generated by excision of a 2.2kbp BamH I fragment from pLYP-2.2. The details for the preparation of the ß-actin probe are described elsewhere (12).

RNase-protection analysis: An in vitro transcribed 200 nucleotides long lyn-RNA transcript (13) was hybridized to 10μg total RNA at 50°C overnight under conditions already described (14).

RESULTS AND DISCUSSION

HL60 cells which were obtained from the American Type Culture Collection at passage number 19 were stimulated to differentiate to macrophage-like, monocyte-like and granulocyte-like cells by using phorbol 12-myristate 13-acetate, 1,25 $[OH]_2$ D_3 or retinoic acid. The final concentration for PMA in the culture medium was 60ng/ml, and those for 1,25 $[OH]_2$ D_3 and retinoic acid were 10^{-7}M and 10^{-6}M, respectively. In all Northern blot analyses, we could detect the lyn-transcript in proliferating, uninduced HL60 cells by using the recloned, purified BamH 1 cDNA insert of the plasmid pLYP-10 (15). Inducing these cells by PMA along the macrophage-like differentiation pathway resulted in a modest increase in the steady-state level of the lyn-transcript after 12 and 18hrs (Fig.1A). Shorter induction periods with PMA ranging from 30min to 6hrs showed similar increased steady-state levels (data not shown). In all experiments neither a significant change in cell viability nor a decrease in the rate of overall protein synthesis was observed (12). Recent studies by ourselves have shown that this stimulation was long enough to observe differentiation events such as the complete depression of

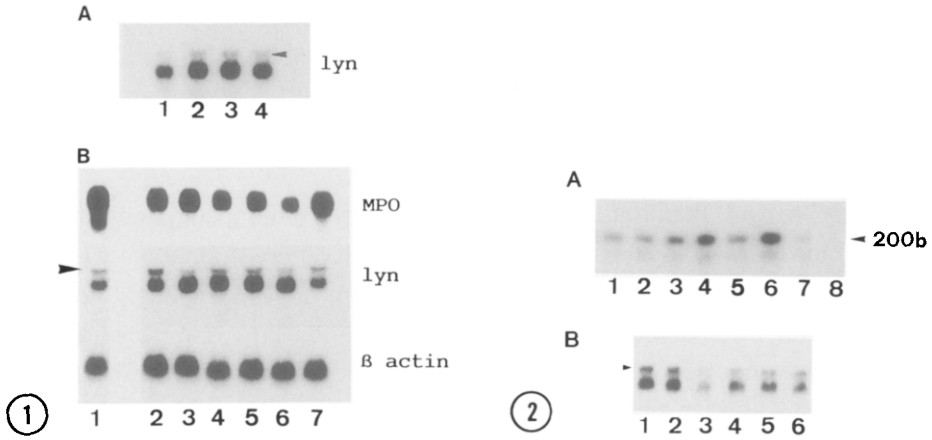

Fig. 1. Moderate induction of lyn-RNA in HL60 cells induced to macrophage- & monocyte-like cells by PMA & 1,25 $[OH]_2$ D_3. (**A**) Cells were induced by 60ng/ml PMA and RNA was extracted at 12hrs (lane 2), 18hrs (lane 3) and 24hrs (lane 4). 10μg total RNA of stimulated cells and of uninduced control cells (lane 1) were electrophoretically separated in a 1% agarose gel and analysed after blotting to a Nytran membrane. Increased levels of lyn-RNA could be detected at all time points investigated within the period of 24hrs.
(**B**) The kinetics for the regulation of the MPO- and the lyn-transcript during 1,25 $[OH]_2$ D_3 induced cell differentiation were established between 17 hrs and 5 days after addition of this inducer. At all time points elevated levels for lyn-RNA were detected compared to control cells. In parallel MPO-RNA levels were partially down-regulated, whereas ß-actin RNA was constitutively expressed. Lane (1 & 7): uninduced control cells lysed at the beginning & at the end of the experiment. Lane 2,3,4,5 and 6: 17hrs, 3d, 3.5d, 4d and 5days after addition of 1,25$[OH]_2$ D_3 to the medium. The arrowheads indicate the second lyn-transcript of 3.7kb found in proliferating as well as in stimulated HL60 cells. The major transcript for lyn had an estimated size of 3.1kb.

Fig. 2. (**A**) RNase-protection analysis with 1,25 $[OH]_2$ D_3 stimulated cells confirmed the moderate increase in lyn-RNA levels seen in Northern blots (Fig. 1B). Lane 1 & 7: RNA from control cells at 0d & 5d after culturing the cells. Lane 2,3,4,5, and 6 represent RNA isolated after 17hrs, 3d, 3.5d, 4d and 5days after cell induction. Lane 8:10μg calf tRNA. (**B**) HL60 cells induced by retinoic acid to granulocyte-like cells. The steady-state lyn-RNA levels were down-regulated but were still detectable after 5 days of cell differentiation. Lane 1: control RNA. Lane 2: 8hrs, lane 3: 24hrs, lane 4, 5, 6: 3d, 4d, 5d after addition of retinoic acid (10^{-6}M) to the cells. The arrowhead points to the 3.7kb lyn-transcript.

myeloperoxidase (11) and neutrophil elastase (data not shown) or the induction of the integrin subunit CD18 (12). Induction of cell differentiation by 1,25 $[OH]_2$ D_3 was extended over a time period of 5 days as even after 5 days exposure this inducer could only partially depress myeloperoxidase (Fig.1B). This differentiation pathway also exhibited an increase in the steady-state levels for lyn-RNA. Already by 17hrs after addition of the steroid, the intracellular concentration of the transcript was higher compared to control cells increasing further by 4 days after stimulating the cells (Fig. 1B). This induction of the lyn-RNA seen in Northern blotting could be confirmed by RNase protection analysis (Fig. 2A). Retinoic acid acting as inducer to granulocyte-like cells showed only small effects on lyn-RNA expression in HL60 cells at the indicated concentration (Fig.2B).

In all Northern blots we prepared, we observed two hybridisation signals in total RNA of HL 60 cells which cannot be explained by the known length dimorphism of 63 nucleotides in the murine lyn mRNA molecules (16). The estimated length of this second minor human lyn transcript was 3.7kb (see Fig.1B and 2B).

Of the eight known members of these src-related protooncogenes seven were investigated for their expression in TPA-stimulated HL60 cells. Only the transcripts of fyn and lyn could be identified under these conditions (17). Intriguingly, fgr and hck transcripts which were detected in mature macrophages (18) and peripheral blood-derived human monocytes (19), respectively, were not identified in TPA-induced HL60 cells. Even 1,25 $[OH]_2$ D_3 which has been claimed to cause differentiation of HL60 cells to a morphologically more monocyte-like cell (17) could not induce hck in these cells. Whereas the src-related protein tyrosine kinases were suggested to be expressed in differentiated cells with little growth potential (20), these kinases seem now to be regulated independently from each other and some of them are predominantly expressed in distinct cell lineages and during specific differentiation stages (18,20,21,22).

In our studies of lyn expression, we showed that even proliferating HL60 cells expressed the lyn-transcript. We observed a modest up-regulation of lyn-RNA in PMA-stimulated cells. Significantly, the level of transcript in HL60 cells differentiated toward granulocytes by retinoic acid was not as much lowered as previously described (17). Our observations indicate an up-regulation of the lyn-transcript during monocyte- and macrophage-like differentiation. This increase in the RNA-level might reflect an increase of its enzymatic activity during these differentiation pathways. So far, two src-like tyrosine kinases (lyn, fyn) have been shown to be expressed in TPA-stimulated HL60 cells (17). As we could detect relatively high levels of the lyn-transcript even in control HL60 cells, it remains puzzling whether src-like genes might play a significant role during TPA-stimulated differentiation of HL 60 cells.

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REFERENCES

1. Hunter, T. & Cooper, J.A. Annu. Rev. Biochem. **54**, 897-930.
2. Metcalf, D.(1989) Nature **339**, 27-30.
3. Clark, S.C., Kamen, R. (1987) Science **236**, 1229-1237.
4. Sherr, C.J. (1988) Biochem. Biophys. Acta **948**, 225-243.

5. Evans J.P.M., Mire Sluis, A.R., Hoffbrand, V.,Wickermassinghe,R.G. (1990) Blood **75**, 88-95.
6. Isfort, R., Huhn, R.D., Frackelton, A.R., Ihle, J.N. (1988) J. Biol. Chem. **263**, 19203-19209.
7. Charbonneau, H., Tanks, N.K., Walsh, K.A., Fisher, E.H. (1989) Proc. Natl. Acad. Sci. USA **86**, 8959-8963.
8. Taetle, R., Ostergaard, H., Smedsrud, M. & Trowbridge, I. (1991) Leukemia **5**, 309-314.
9. Frank, D.a., Sartorelli, A.C. (1986) Biochem. Biophys. Res.Commun. **140**, 440-447.
10. Huang, M.M., Bolen, J.B., Barnwell, J.W., Shattil, S.J. & Brugge J.S. (1991) Proc. Natl. Acad. Sci. USA **88**, 7844-7848.
11. Meier, R.W., Chen, T., Friis, R.R. & Tobler A. (1991) Biochem. Biophys. Res. Commun. **176**, 1345-1350.
12. Meier, R.W. et al. submitted.
13. Bielke, W. et al. Manuscript in preparation.
14. Gilman, M. in Current Protocols in Molecular Biology Volume I, eds.: Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K.; John Wiley & Sons.
15. Yamanashi, Y., Fukushige, S.-I., Semba, K., Sukegawa, J., Miyajima, N., Matsubara, K.-I., Yamamoto, T. & Toyoshima, K. (1987) Mol. Cell. Biol. **7**, 237-243.
16. Stanely, E., Ralph, S., Mc Ewen, S., Boulet, J., Holtzman, D.A., Lock, P., Dunn, A.R. (1991) Mol. Cell. Biol. **11**, 3399-3406.
17. Katagiri, K., Katagiri, T., Koyama, Y., Morikawa, M., Yamamoto, T., Yoshida, T. (1991) J. Immunol. **146**, 701-707.
18. Ley, T. J., Connally, N.L., Katamine, S., Cheah, M.S.C., Senior, R.M. & Robbins K.C. (1989) Mol. Cell. Biol. **9**, 92-99.
19. Eisenman, E., & Bolen, J.B. (1990) Cancer Cells **2**, 303-310.
20. Yamanashi, Y.S., Mori, M., Yoshida, T., Kishimoto, K., Inoue, T., Yamamoto, Toyoshima, K. (1989) Proc. Natl. Acad. Sci. USA **86**, 6538-6542.
21. Marth, J.D., Lewis, D.B., Wilson, C.B., Gearn, M.E., Krebs, E.G. & Perlmutter, R.M. (1987) EMBO J. **6**, 2727-2734.
22. Ziegler, S.F., Marth, J.D., Lewis, D.B. & Perlmutter, R.M. (1987) Mol. Cell. Biol. **7**, 2276-2285.