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CHANGES OF C-MOS EXPRESSION IN RESPONSE TO 2-O-TETRADECANOYLPHORBOL-13-ACETATE IN UNDIFFERENTIATED TERATOCARCINOMA CELLS

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Summary: Treatment of the cells of the 311 cell line, a pluripotent mouse teratocarcinoma cell line, with 12-O-tetradecanoylphorbol-13-acetate (TPA) modulates c-mos expression. Transient suppression of 6.1 and 4.6 kilobases (kb) transcripts and activation of 1.8 transcript indicate that TPA mediates concurrently positive and negative regulation of c-mos transcription.

The results show that the c-mos gene is a TPA-modulated gene. In addition, a TPA-responsive element (GTGACTCA), which exists in the 5'-flanking region of c-mos gene of Balb/c mice [1,2], is suggested to be involved in this response.

However, these changes were not accompanied by early marker changes associated with endodermal cell differentiation, i.e., morphological change, induction of plasminogen activator and suppression of glucose transport activity.

Proto-oncogene c-mos , the cellular homolog of the transforming gene of Molony murine sarcoma virus, is characterized by the lack of expression in a variety of differentiated tissues, possibly because of the existence of an inhibitory upstream sequence [2], and by stage-specific expression during early embryo development [3] and in undifferentiated teratocarcinoma(EC) cells [4]. Thus, the c-mos product may play a role in early stages of development.

On the other hand, Protein kinase C, known to be activated by TPA, is important in transduction of signals and might be involved in cell differentiation in early development. Since several proto-oncogenes, including differentiation-linked genes, are also TPA-responsive [5,6,7], the c-mos gene may also be regulated by a common mechanism and be responsive to TPA. Specific regulatory proteins whose activity or abundance is modulated by protein kinase C and which interact with distinct cis-acting elements [8,9,10], have been proved to be involved in the action mechanism of TPA to activate the transcription of genes. These elements contain a short nucleotide sequence that is highly conserved in a number of TPA-inducible

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promoters and one of which, GTGACTCA, is referred to as the TPA responsive element (TRE). The existence of TRE in the 5'-flanking region of c-mos gene of Balb/c mice [1,2] promoted us to test the TPA-responsiveness of c-mos expression.

Here we report that c-mos gene is also TPA-modulated.

Materials and Methods

Cell line and culture conditions:

A pluripotent teratocarcinoma cell line 311 was isolated by serial cloning on a feeder layer from a spontaneous testicular teratocarcinoma from a 129 mouse and was cultivated as previously described. Briefly, 311 cells from stock culture on feeder cells were seeded at 10^6 cells per 60 mm gelatin-coated tissue culture dish in the medium supplemented with 10^{-4}M 2-mercaptoethanol [11]. After 2 days, cells were detached and replated at an appropriate cell density in the same manner as above. After 2 days, the cultures were treated with 50nM TPA for the designated hours.

Measurement of 3-O-methylglucose uptake:

The uptake was initiated by the addition of 1ml of phosphate-buffered saline (PBS) containing 3-O-methylglucose (4μM,0.1μCi/ml) at 20°C. After 2min, hexose uptake was stopped by washing with 2ml of ice-cold PBS and cells were denatured with 5% trichloroacetic acid and then dissolved in 0.9 ml of 0.1M NaOH/0.1% sodium dodecyl sulfate (SDS), and aliquots of the lysate were taken for assay of radioactivity and for protein determination. Carrier-mediated uptake was calculated after correction for non-specific uptake determined with L-glucose.

Plasminogen activator(PA) assay:

The cultures were assayed for the production of plasminogen activator(PA) by an agar-overlay assay as previously described [11].

Isolation of RNA and northern blot analysis:

Total cellular RNA was isolated [12] and quantified by absorbance at 260 nm. Quantification was confirmed by electrophoretical fractionation of a sample on formaldehyde-agarose gels and transfer to nitrocellulose, probing with actin DNA, or staining rRNA bands with methylene blue as described previously [4]. Total RNAs from each indicated cell were electrophoresed on a formaldehyde gel [13], transferred to nitrocellulose [14] and hybridized with nick translated probes [15]. Each filter was washed at 55°C in 0.1XSSC (15mM NaCl,1.5mM Na Citrate), 0.5%SDS and exposed to Kodak X-OMAT film. The size of c-mos transcripts were determined relative to 18 and 28 S rRNA markers, which were assumed to be 2.0 and 5.1 kb, respectively.

Probes:

The plasmids or the fragments containing cloned retroviral genome and actin gene were used as specific probes: mos [1]; human cardiac muscle actin gene [16].

Results

Modulation of c-mos gene in response to TPA:

We observed the transient suppression in 6.1 and 4.6 kb transcripts in contrast to the activation in 1.8 kb transcripts of the pluripotent teratocarcinoma cell line, 311, in response to TPA (Fig.1). Transcriptional modulation of the

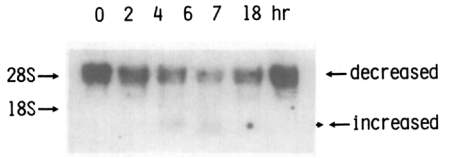

Figure 1. Effects of TPA treatment on c-mos expression. Cell line 311 cells were cultured as described in Materials and Methods. Total RNAs(14 μg) isolated from the cells were electrophoresed, transffered to nitrocellulose and hybridized to SacI-HindIII fragment of c-mos gene cloned to pBR322 (pMS1). Hybridization to XbaI-AvaI fragment or AvaI-HindIII fragment of c-mos gene gave the same results. RNAs from the cells stimulated for 0, 2, 4, 6, 7 and 18hr with TPA.

gene was detectable within 2hr of TPA addition, but the change is temporary, returning to the initial level within 7 hr. No further changes in c-mos transcription were observed by additional treatment with TPA. Furthermore, the results indicate that the dual modulation occurs concertedly.

Activation of c-mos gene without DNA rearrangement:

An upstream inhibitory sequence (UMS) prevents c-mos expression and deletion of this region has been reported to result in its activation [2]. We attempted to determine whether c-mos transcripts result from the aberrant DNA. UMS is known to be deleted commonly by DNA rearrangement at the rc-junction [17,18,19] (indicated by the arrow in Fig.2) resulting in rc-mos. Namely, rc-junction is the site where novel DNA fragment is inserted within the c-mos sequence.

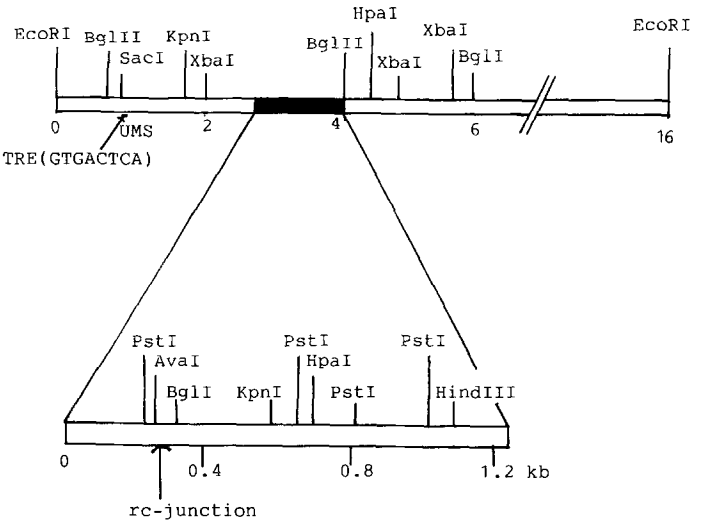

Figure 2. Physical map of c-mos gene of Balb/c mouse.
UMS: upstream mouse sequence
TRE: TPA-responsive element (GTGACTCA)
closed rectangle: the coding sequence for c-mos gene

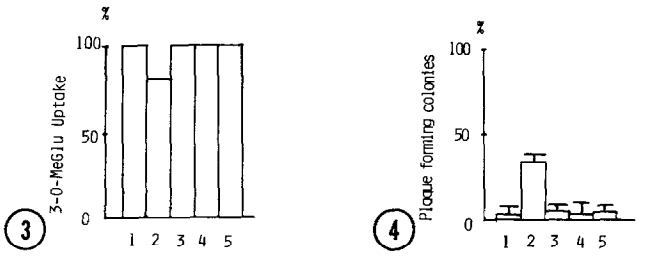

Figure 3. Effect of TPA on 3-O-methylglucose uptake. At the end of cultivation, 3-O-methylglucose uptake for 2min was assayed. 4-, 6-, and 18-hr culture with TPA (lane 3,4,5); 4-day culture with (lane 2) and without RA (lane 1)

Figure 4. Effects of TPA on PA production. The cells were cultured with or without TPA or RA. PA production was estimated by the formation of caseinolytic plaques, as described in Materials and Methods, and was expressed as the percentage of the colonies which formed plaque. No proteolytic plaques were observed in the absence of plasminogen, which shows that digestion of casein was due to plasminogen activator secretion. 4-, 6-, and 18-hr culture with TPA (lane 3,4,5); 4-day culture with (lane 2) and without RA (lane 1)

Since the AvaI site is located at the site 5' to the rc-junction, the XbaI-AvaI fragment might hybridized with trannscripts resulted from c-mos but not from rc-mos gene. All transcripts (Fig. 1), when cultured with or without TPA, were hybridize with the XbaI-AvaI fragment of the c-mos gene, which suggests that c-mos itself was activated.

The activity of TPA to induce EC cell differentiation:

To determine the relationship between transient changes of c-mos expression induced by TPA and EC cell differentiation, changes in several differentiation markers were examined, i.e., changes in the morphology of cells and the secretion of plasminogen activator (PA). These properties are commonly used as markers in the study of differentiation of teratocarcinoma cells in vitro [20]. 3-O-Methylglucose uptake was also shown to associate with EC cell differentiation [21]. Changes of these differentiation markers were not observed during the change of c-mos expression; the level of the uptake of 3-O-methylglucose (Fig.3), PA production (Fig.4) and morphology did not change during the 1- to 18-hr treatment with TPA, in contrast to the decreased uptake of 3-O-methylglucose, the increased percentage of PA-producing colonies and the appearance of endodermal cells in the presence of RA.

Discussion

Propst and Vande Woude reported the tissue-specific regulation of the size of the mos transcripts. We also reported three major transcripts in undifferentiated EC cells of the pluripotent cell line 311. Three transcripts may be independently regulated, although all three transcripts were coincidently suppressed when cell differentiation was induced by RA [4]. The results that TPA mediates positive regulation in one transcript and negative regulation in others supports Propst's proposal that each c-mos transcript is independently regulated and may play a different role.

TPA treatment, known to stimulate protein kinase C which is important in transduction of signals generated at the plasma membrane, leads to induce of various proto-oncogenes and several other genes. Analysis of several TPA inducible genes revealed a consensus sequence which functions as a TPA responsive element (TRE) conferring inducibility to heterologous promoters. This short nucleotide sequence is highly conserved in a number of other TPA-inducible promoters and the binding site for a trans-acting factor (AP-1) whose activity is regulated by TPA. Since 129 mouse, from which the 311 cell line was derived, is shown to share with Balb/c mouse an identical 16 kb EcoRI fragment including c-mos gene , the region from the EcoRI site at 0 kb to the EcoRI site at 16 kb on the map by Southern blot analysis [4], TRE (5'-GTGACTCA-3') which exists in the 5'-flanking region of the c-mos gene in Balb/c mice [1,2], may also exist in the 311 cells. Therefore, this sequence may be one of the elements that mediate modulation of c-mos transcription. However, we cannot exclude the possibility that the change in the quantity of mRNA results from the degradation of the 6.1 and 4.6 kb to the 1.8 kb transcripts.

Among the transcription factors which recognize the same sequence as TRE [9,10,22,23,24], PEA1 is reported to be detected not in undifferentiated but in differentiated EC cells segregating during in vitro differentiation [22], implicating that TRE may play a role in EC cell differentiation and that the response of c-mos transcripts to TPA is also involved in EC cell differentiation.

Since TPA is known to induce cell differentiation in several types of cells [25,26] and c-mos suppression in EC cells is accompanied by EC cell differentiation, TPA may trigger EC cell differentiation. However, the lack of effect suggests that this is not the case, i.e., such TPA-induced modulation of the c-mos gene is not involved in EC cell differentiation. However it seems more likely that the modulation of the c-mos gene is one of the earliest observed events and is by itself insufficient to commit the 311 cells to differentiate to endoderm-like cells. We assume that this response is one of the earliest events elicited by physiological signals which induce EC cell differentiation and that TPA-treatment only mimics some early steps of cell differentiation.

The molecular mechanisms of this response is unknown, and further study is required to understand the mechanism of action of TPA and the c-mos product in cell differentiation.

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References

1. Blair, D.G., Oskarsson, M., Wood, T.G. McClements W.L., Fishinger, P.J. and Vande Woude, G.F. (1981). Science 212, 941-943
2. Wood, T.G., McGeady, M.L., Baroudy, B.M., Blair, D.G. and Vande Woude, G.F. (1984) Proc. Natl. Acad. Sci. USA 81, 7817-7821
3. Propst, F and Vande Woude, G.F. (1985) Nature 315, 516-518
4. Ogiso, Y., Matsumoto, M., Morita, T., Nishino, H., Iwashima, A., and Matsushiro, A. Biochem. Biophys. Res. Commun. 140, 477-484
5. Kelly, K., Cochran, B.H., Stiles, C.D and Leder, P. (1983) Cell 35, 603-610
6. Greenberg, M.E. and Ziff, E.B.(1984) Nature 311,433-438
7. Colamonici, O.R., Trepel, J.B. Vidal, C.A. and Neckers, L.M. (1986) Mol. Cell Biol. 6, 1847-1850
8. Angel, P., Baumann, I., Stein, B., Delius, H., Rahmsdorf, H.J. and Herrlich, P. (1987) Mol. Cell Biol. 7, 2256-2266
9. Lee, W., Mitchell, P. and Tijian, R. (1987) Cell 49, 741-752
10. Angel, P., Imagawa, M. Chiu, R., Stein, B., Imbra, R.J., Rahmsdorf, H.J., Jonat, C., Herrlich, P. and Karin, M. (1987) Cell, 49, 729-739
11. Ogiso, Y., Kume, A., Nishimune, Y. and Matsushiro, A. (1982) Exp. Cell Res. 137, 365-372
12. Chirgwin, J.M., Przybla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochem. 18 5294-5299

13. Ono, M., Cole, M.D. White, A.T. and Huang, R.C.C. (1980) Cell 21, 465-473
14. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517
15. Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201-5205
16. Hamada, H., Petrino, M.G. and Kakunaga, T. (1982) Proc. Natl. Acad. Sci. USA 79, 5901-5905
17. Rechavi, G., Givol, D. and Canaani, E (1982) Nature 300, 607-611
18. Gattoni-Celli, S., Hsiao, W.L.W and Weinstein, I.B. (1983) Nature 306, 795-796
19. Canaani, E., Dreazen, O., Klar, A., Rechavi, G., Ram, D., Cohen, J.B. and Givol, D. (1983) Proc. Natl. Acad. Sci. USA 80, 7118-7122
20. Strickland, S. and Mahdavi, V. (1978) Cell 15,393-403
21. Ogiso, Y., Kitagawa, K., Nishino, H., Iwashima, A., and Shudo, K. (1987) Exp. Cell Res. 173, 262-266
22. Kryszke, M.H., Piette, J. and Yaniv, M.(1987) Nature 328, 254-256
23. Imler, J.L., Schatz, C., Wasylyk, C., Chatton, B. and Wasylyk, B. (1988) Nature 322, 275-278
24. Bos, T.J., Bohmann, D., Tsuchie, H., Tijian, R. and Vogt, K (1988) Cell 52, 705-712
25. Kreutter, D., Caldwell, A.B. and Morin, M.J. (1985) J. Biol. Chem. 260, 5979-5984
26. Kraft, A.S., Smith, J.B. and Berkow, R.L. (1986) Proc. Natl. Acad. Sci. USA 83, 1334-1338