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Induction of specific protein tyrosine phosphatase transcripts during differentiation of mouse embryonal carcinoma (F9) cells

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Abstract

We have investigated the pattern of PTPase transcript expression during in vitro differentiation of mouse embryonal carcinoma (F9) cells. While the transcripts of most PTPases were unchanged or undetected during embryonal differentiation induced by retinoic acid, several PTPase transcripts exhibited distinct patterns of induction. Mutant cells defective in differentiation did not display the induction of some of these PTPase transcripts. Interestingly, three out of the four PTPase transcripts induced were the same PTPase transcripts induced during in vitro erythroid differentiation of mouse erythroleukemia (MEL) cells [(1982) Biochem. Biophys. Res. Commun. 107, 1104–1109]. The possible role played by specific PTPases in cell differentiation is discussed.

Key words: Differentiation; Protein tyrosine phosphatase; Embryonal carcinoma cell

1. Introduction

F9, an established mouse embryonal carcinoma cell line, differentiates into primitive endoderm-like cells in vitro when treated with retinoic acid [1]. Dibutyryl cyclic AMP (dbcAMP) treatment further differentiates the primitive endoderm-like cells into those which exhibit characteristics of parietal endoderm cells [2,3]. The endoderm-like cells can also be converted to visceral endoderm-like cells when exposed to cell-aggregation conditions [3]. Because of these distinct characteristics of differentiation, F9 cells have been widely used as a model for studying the mechanism of embryonic differentiation.

We have shown previously that inhibitors of protein tyrosine kinases, such as herbimycin A, induce F9 differentiation like retinoic acid [4], suggesting that the dephosphorylation of phosphorylated tyrosine moieties of cellular proteins is involved in the differentiation process. Possible involvement of tyrosine dephosphorylation has been studied more extensively in erythroid differentiation of mouse erythroleukemia (MEL) cells, in which not only a series of inhibitors of protein tyrosine kinases induce differentiation, but also phosphotyrosine moieties of cellular proteins were rapidly dephosphorylated at a very early stage of differentiation [4–9]. Na_3VO_4, a specific inhibitor of protein tyrosine phosphatases (PTPases) [10], has been shown to prevent tyrosine dephosphorylation as well as erythroid differentiation [8]. More recently, we have found that the quantitative level of transcripts for three specific PTPases is sharply increased at a very early stage of differentiation [11].

In order to explore the possible involvement of protein tyrosine dephosphorylation in differentiation, we examined the level of transcripts of sixteen known and recently cloned PTPases during F9 cell differentiation. Here we report that while the transcripts of most of the PTPases were unchanged or undetected, several specific PTPase transcripts were induced during embryonal differentiation induced by retinoic acid. Mutant F9 cells defective in differentiation did not show the induction of some of the PTPase transcripts. Comparing the patterns of expression of these PTPase transcripts during F9 cell differentiation with those during erythroid differentiation of MEL cells, the possible roles of PTPases in cell differentiation is discussed.

2. Materials and methods

2.1. Materials

Retinoic acid (RA) and dibutyryl cyclic AMP (dbcAMP) were purchased from Sigma. ES medium was purchased from Nissui Seiyaku (Tokyo). Fetal calf serum (FCS) was obtained from United Biotech. [α-^{32}P]dCTP was purchased from ICN. All other agents were reagent grade.

2.2. Cells and cell culture

F9 cells were supplied by Dr. Y. Nishimune. Differentiation-defective mutant cell lines (RA^r-6 and ROT^r-1) were isolated from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-treated F9 cells. The cells were cultured at 37°C in a CO_2 (5%) incubator in ES medium supplemented with FCS (10%). Plastic tissue culture dishes had been coated with gelatin solution (0.4%) before use.

2.3. Assay for plasminogen activator (PA)

Plasminogen activator was assayed as described by Nishimune et al.

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[12]. In essence, F9 cells were overlaid with ES medium containing Noble agar (0.75%, w/v), skimmed milk (2.5%, w/v), and human plasminogen (0.2 casein U/ml). After 24 h of incubation at 37°C in a CO_2 incubator, haloes were counted. In a standard assay, over 200 colonies per plate (in duplicate) were examined.

2.4. Northern blot analysis

Exponentially grown F9 cells were exposed to retinoic acid (1 μM) or reteinoic acid (1 μM) plus dbcAMP (1 mM). At different time intervals the cells (~2×10^8 cells) were collected by a rubber policeman, washed with cold PBX (3 times), lysed in 2.4 ml of RNA extraction buffer (140 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.6, and 0.5% Nonidet P40) and total RNA was prepared as described [13]. Poly(A)$^+$ RNA was obtained from the total RNA preparations using Oligotex-dT30 (Takara Shuzo) according to the manufacturer's instruction.

For Northern blotting, poly(A)$^+$ RNA was electrophoresed on a 1% formaldehyde-agarose gel, blotted onto a Hybond N$^+$ membrane (Amersham), and hybridized with ^{32}P-labeled PTPase cDNA probes which were prepared by random priming (Boehringer-Mannheim) using [α-^{32}P]dCTP. The specific activities of the probes were approximately 2×10^9 cpm/μg DNA. The hybridization was carried out overnight at 42°C in a hybridization buffer (50% fomamide, 5 × SSC, 50 mM Tris-HCl, pH 7.5, 0.1% SDS and 0.1 mg/ml sonicated herring sperm DNA). The membranes were washed once with 2 × SSC, 0.1% SDS for 10 min at room temperature, once with 2 × SSC, 0.1% SDS for 15 min at 65°C, twice with 0.1 × SSC, 0.1% SDS for 15 min at 65°C and autoradiographed using Kodak X-OMAT films (exposure for overnight).

3. Results and discussion

We first examined whether the transcription of any specific PTPase transcripts was induced or the quantitative level of transcripts was altered during F9 cell differentiation. The cells were incubated in the presence of retinoic acid (for primitive endoderm differentiation) or retionic acid plus dbcAMP (for parietal endoderm differentiation). Poly (A)$^+$ RNAs were prepared from the cells at different time intervals up to 96 h. Under the conditions employed, both primitive and parietal endoderm differentiation were completed by approximately 72 h of incubation, and the cellular commitment to differentiation is thought to occur much earlier, by 24–48 h of incubation. The RNAs were then subjected to Northern blot analysis using PTPase probes. For the probes, we used sixteen different mammalian PTPase cDNA clones which included many of the recently cloned PTPase cDNAs. The cDNA clones used as probes are listed in Table 1.

Table 1
List of PTPase clones

Clone	Reference
CDC25C	Sadhu et al. [14]
Cdc25M2	Kakizuka et al. [15]
CD45	Thomas et al. [16]
LRP	Matthews et al. [17]
MPTP	Mosinger et al. [18]
PEP	Matthews et al. [19]
PTPβ2 (BET)	Thomas et al. [20]
PTPδ	Thomas et al. [20]
PTPε	Thomas et al. [20]
PTPκ (MBR)	Thomas et al. [20]
PTPμ	Gebbink et al. [21]
PTP-S	Swarup et al. [22]
RIP (MTC)	Thomas et al. [20]
SHP	Matthews et al. [19]
SH-PTP2	Freeman et al. [23]
STEP	Lombroso et al. [24]

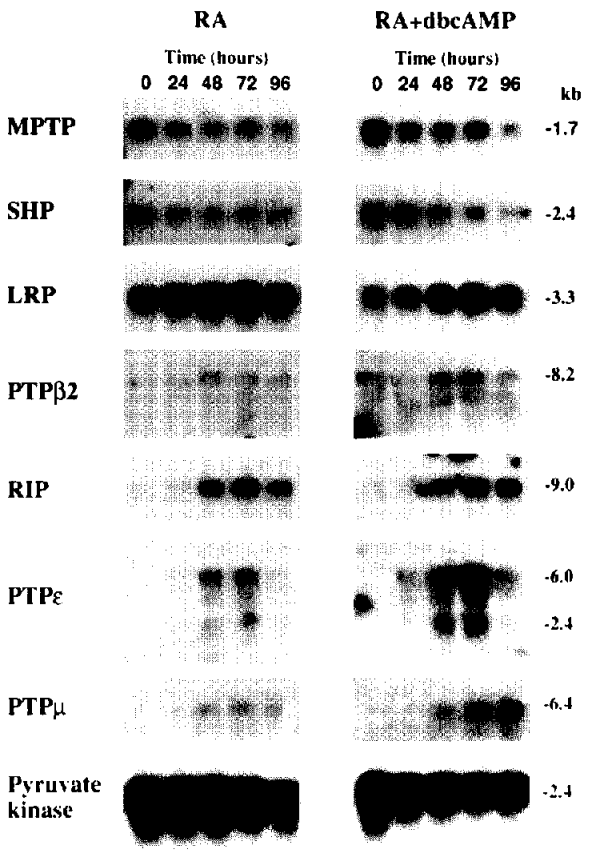

Fig. 1. Alteration of PTPase transcripts during F9 cell differentiation. Exponentially grown F9 cells were exposed to retinoic acid (1 μM) or retinoic acid (1 μM) plus dbcAMP (1 mM). Cells (~2×10^8) were collected at 0, 24, 48, 72 and 96 h of incubation with the agents, and poly(A)$^+$ RNA was prepared. The RNA (2 μg) was then subjected to Northern blot analysis using various ^{32}P-labeled PTPase and control (pyruvate kinase) cDNA probes.

Transcripts of thirteen PTPases out of the sixteen examined were detected in control (untreated) cells or induced cells or both. In Fig. 1, we show the patterns of expression of seven PTPase transcripts, representatives of the PTPase transcripts detected up to 96 h after the addition of retinoic acid or retinoic acid plus dbcAMP. They were roughly classified into several groups according to their pattern of altered expression during differentiation. Some of the PTPase transcripts, exemplified by MPTP in the figure, remained at relatively constant levels up to 72 h after treatment of the cells with retinoic acid as well as retinoic acid plus dbcAMP. The other transcripts which behaved similarly to MPTP transcripts included those for CDC25C, Cdc25M2, PTPκ (MBR), PTP-S, SH-PTP2 and STEP (data not shown). In the

96 h samples the quantitative level of these PTPase transcripts and most of the others were found to be significantly lower, probably as a result of prolonged incubation of differentiated cells.

In contrast to these PTPase transcripts which remained unchanged during F9 differentiation, six PTPase transcripts exhibited alteration in their levels of expression (Fig. 1). Among them, the level of SHP transcripts gradually decreased, particularly in the cells treated with retinoic acid plus dbcAMP. LRP transcripts were present at a significantly high level in control cells and the level increased gradually (2–3 fold) during differentiation. In contrast to these PTPase transcripts, four PTPase transcripts (PTPβ2, RIP, PTPε, PTPμ) either were not detected or existed at a very low level in the untreated cells, but their levels increased substantially starting at between 24 and 48 h incubation with retinoic acid or retinoic acid plus dbcAMP, suggesting that these PTPases are involved in F9 differentiation.

The experiments shown in Fig. 1 also indicate that no significant differences, with the possible exception of SHP transcripts, were observed between the patterns of expression of the PTPase transcripts in the cells treated with retinoic acid and those treated with retinoic acid plus dbcAMP. We also examined the patterns of expression of the PTPase transcripts during F9 differentiation to visceral endoderm-like cells when induced by aggregation of the cells in the presence of retinoic acid. The results were essentially the same as those observed for cells treated with retinoic acid or retinoic acid plus dbcAMP (data not shown). Since differentiation to parietal endoderm-like cells (induced by retinoic acid plus dbcAMP) and to visceral endoderm-like cells (induced by aggregation in the presence of retinoic acid) is via primitive endoderm-like cells (induced by retinoic acid), the results suggest that alteration of the patterns of expression of specific PTPase transcripts reflects a cellular response to differentiation to primitive endoderm-like cells, and not to the subsequent differentiation to parietal or visceral endoderm-like cells.

In order to obtain further evidence for the relationship between the increased quantitative level of the transcripts for PTPβ2, RIP, PTPε and PTPμ and F9 differentiation,

Table 2
Plasminogen activator production in mutant F9 cells

Cells	Plasminogen activator-positive colonies (%)	
	–	Retinoic acid
Wild-type	0.3	77.1
RA^r-6	0.2	1.7
ROT^r-1	0.3	1.1

Cells were incubated with or without retinoic acid (1 μM) for 2 days and plasminogen activator-positive colonies were assayed as described in section 2.

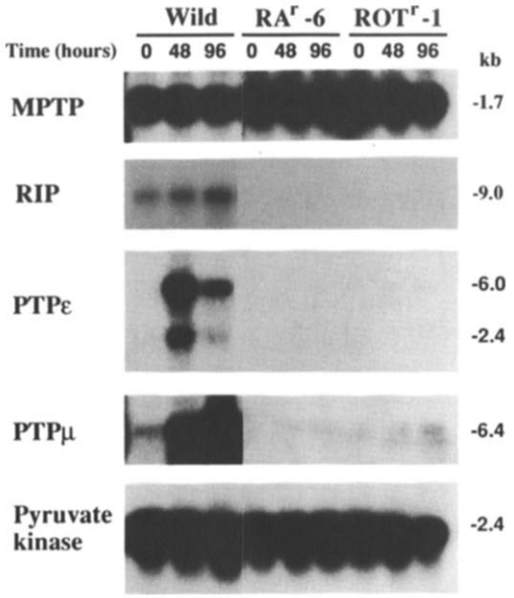

Fig. 2. Induction of MPTP, RIP, PTPε and PTPμ transcripts in differentiation-defective F9 cell mutants. Exponentially grown wild-type F9 cells and differentiation-defective mutant cells RA^r-6 and ROT^r-1 were exposed to retinoic acid (1 μM). At 0, 48 and 96 h of incubation with retinoic acid, cells ($\sim 2 \times 10^8$) were collected and poly(A)$^+$ RNA was prepared. The RNA (3 μg) was then subjected to Northern blot analysis using ^{32}P-labeled MPTP, RIP, PTPε, PTPμ, and control (pyruvate kinase) cDNA probes.

we examined the expression level of these PTPase transcripts (plus MPTP as a positive control) in differentiation-defective mutant F9 cells (RA^r-6 and ROT^r-1). These mutant F9 cells were independently isolated and are defective at least for both primitive endoderm and parietal endoderm differentiation [25]. The loss of plasminogen activator production, an indication of primitive endoderm differentiation, of these mutant cells is shown in Table 2. The mutant cells were incubated in the presence of retinoic acid, and poly(A)$^+$ RNAs isolated at different time intervals were subjected to Northern blot analysis as performed earlier. The results are shown in Fig. 2. RIP and PTPε transcripts were hardly detected in either of these mutant cells before and after retinoic acid treatment. As for the PTPμ transcripts, they were detected in the mutant cells but the level of expression did not increase significantly as observed in the parental cells after retinoic acid treatment. The pattern of expression of PTPβ2 transcripts in the mutant cells did not differ significantly from that in the parental cells (data not shown). It seems that the transcription of at least three PTPases (PTPε, PTPμ and RIP) was affected by the introduction of the mutation(s) which resulted in defective F9 differentiation. These results further support the view that the induction of specific PTPase transcripts is associated with F9 differentiation, although it is not clear whether the behavior of these PTPase transcripts in the mutant cells is a direct result of the mutations or an

indirect consequence of the defect in differentiation of these mutant cells.

We reported recently that three specific PTPase transcripts (RIP, PTPβ2 and PTPε) among over one dozen known and recently cloned PTPases were induced at an early stage of differentiation in MEL cells induced by DMSO or HMBA [11]. In the experiments presented here, we found that four out of sixteen known PTPase transcripts were apparently induced during F9 differentiation. Among them, RIP, PTPβ2 and PTPε are the ones which also exhibited induction in MEL cell differentiation. The RIP transcripts were either non-detectable or remained at a very low level in mutant MEL cells defective in erythroid differentiation [11]. Although the behavior of these transcripts must be carefully interpreted, the induction of these three specific PTPase transcripts (RIP, PTPβ2 and PTPε) during both MEL and F9 cell differentiation may not be simply coincidental. If this is the case, one could speculate that one (or more) of these specific PTPases plays a common role in the induction of differentiation in two entirely different in vitro differentiation systems, one of which is a terminal differentiation and another an embryonic differentiation.

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