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Differential effects of DNA tumor virus nuclear oncogene products on adipocyte differentiation

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We have introduced SV40 and polyoma large T antigen- and adenovirus-type 12 E1A genes into mouse 3T3-L1 preadipocyte cells to study the ability of various nuclear oncogene products to modulate cell differentiation. Clones expressing E1A products could differentiate into adipocytes faster than the control in spite of the absence of adipogenic inducers, as measured by the appearance of lipid droplets microscopically and by staining accumulated triglycerides with oil red O. However, clones expressing SV40 and polyoma large T antigens could not differentiate even if they were exposed to the inducers.

DNA tumor virus; Nuclear oncogene; Adipogenic differentiation

1. INTRODUCTION

SV40 and polyoma large T antigens and adenovirus E1A products confer on cells in culture the ability to grow in the presence of low concentrations of serum and, in the case of redent primary embryonic cells, an unlimited potential for growth in culture [1]. This phenomenon is explained by the fact that those gene products which are localized in nuclei modulate the expression of the important genes for growth control [2–4]. Adipogenic differentiation of 3T3-L1 cells involves a change from a fibroblastic morphology to an enlarged rounded cell shape with the accumulation of abundant lipid droplets within the cells [5]. Induction of specific sets of proteins as well as hormone receptors occurs along with the morphological changes after treatment of adipogenic inducers. These experimental results raised the question as to whether exogenously transfected genes which can modulate gene expressions are able to induce adipocyte differentiation. We report here the differential activities of the DNA tumor virus oncogene products on preadipocyte differentiation.

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2. MATERIALS AND METHODS

2.1. *Plasmid construction*

pSV3neo plasmid DNA [6] was used as the source of the SV40 large T antigen gene. *Bgl*I-*Bam*HI fragment (4158 bp) of pPyLT [7] containing the polyoma virus large T antigen gene was linked with RSV-LTR after changing a *Bgl*I site to a *Hin*dIII site (pSRPyLT). The fragment having *Bam*HI sites of pSV2neo DNA [6] was inserted at the *Bam*HI site of pSRPyLT DNA as a drug-resistant marker (pSRPyLTneo). *Bam*HI-*Eco*RI fragment (1600 bp) from gAE1A [8] containing an adenovirus type 12 (Ad12) E1A gene was ligated with the *Bam*HI-*Eco*RI cleaved pSV2neo DNA (pSV2neoE1A). The maps of these plasmids are shown in fig.1.

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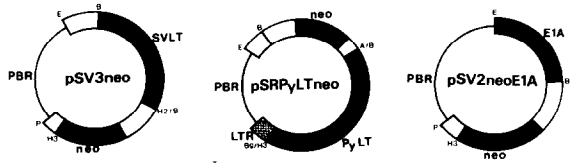


Fig.1. Maps of constructed plasmids containing DNA tumor virus nuclear oncogenes. As a selective marker, the gene conferring G418 resistance indicated by neo (▬) [6] was inserted in all plasmids. For polyoma virus large T antigen gene (PyLT), the DNA replication origin was replaced with RSV-LTR indicated by LTR [3] in order to stabilize the integrated T antigen gene of polyoma virus in mouse 3T3-L1 cells. A, B, Bg, E, H2, H3 and P, represent *Acc*I, *Bam*HI, *Bgl*I, *Eco*RI, *Hin*dII, *Hin*dIII, and *Pvu*II cutting sites, respectively. SVLT, E1A and PBR denote SV40 large T antigen gene, adenovirus E1A gene, and PBR region in the plasmids, respectively.

2.2. *Cell culture conditions*

Mouse 3T3-L1 cells obtained from Dr H. Green (Harvard University, USA) through Dr Y. Kitagawa (Nagoya University, Japan) were maintained in Dulbecco's minimal essential medium (DMEM) containing 10% fetal calf serum. The cells were subcultured every 4–6 days before reaching confluence. Plasmid DNAs were transfected on semi-confluent 3T3-L1 cells as described [9,10]. At 36 h after transfection, G418 was added to the culture medium at a final concentration of 400 μg/ml. G418-resistant small sparse colonies were picked up before they stayed in confluency for a long period. Those clones were cultivated in the presence of G418 and subcultured before becoming confluent.

2.3. *Measuring adipogenic differentiation*

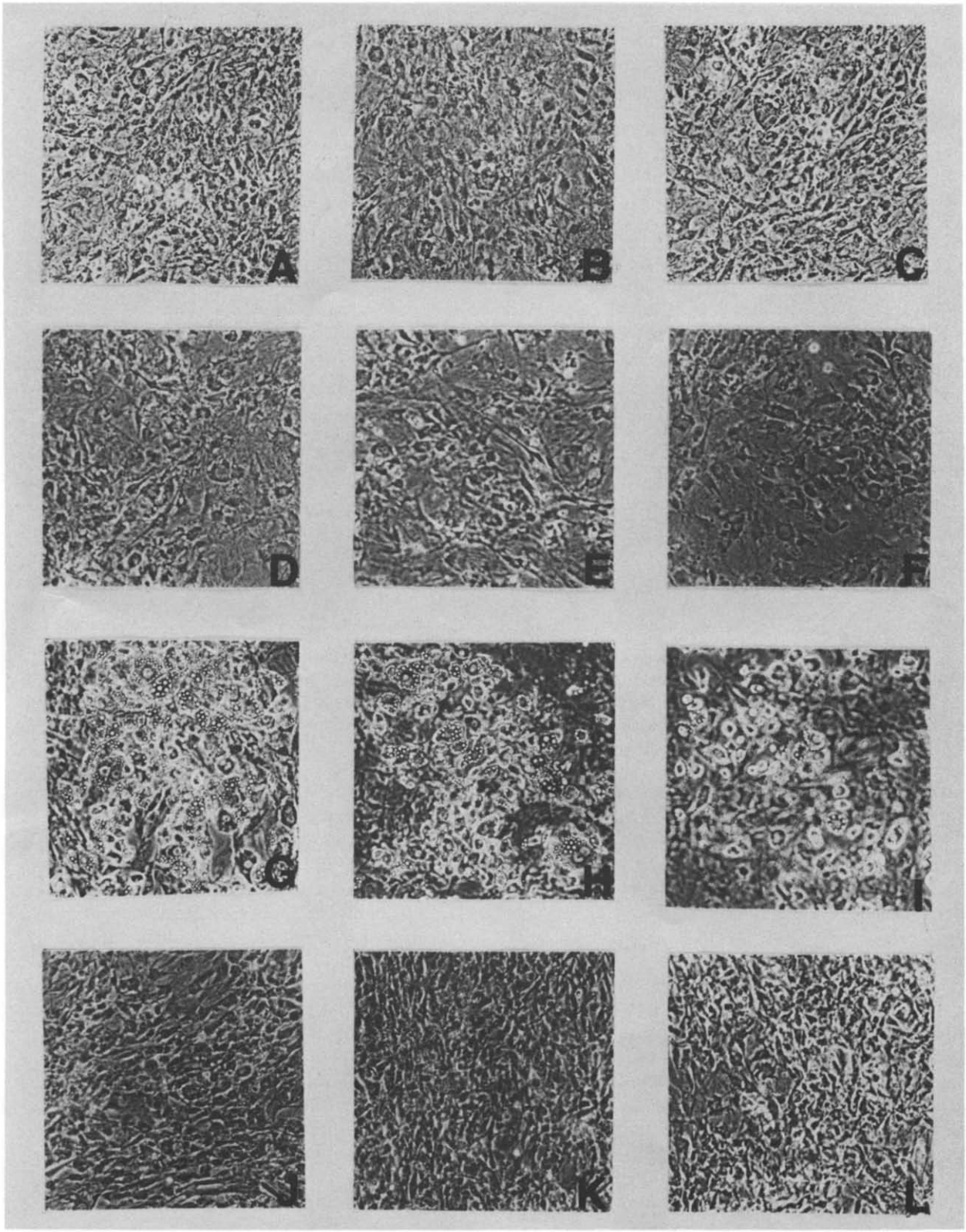
The lipid droplets in the differentiated adipocytes were observed microscopically, and stained with 3 μg/ml oil red O after fixing with 3.3% formaldehyde.

3. RESULTS

Eleven, four, and ten clones were isolated from SV40 and polyoma large T antigen and Ad12 E1A gene-transfected cell cultures, respectively. By fluorescent antibody test and immunoprecipitation, all clones expressed SV40 and polyoma large T antigen and Ad12 E1a product (not shown). Growth rates of E1A- and polyoma large T antigen-expressing cells are about the same as those of parental 3T3-L1 cells and pSV2neo transfected 3T3-L1 clones. On the other hand, SV40 large T antigen-expressing clones showed the typical transformed phenotypes that they could grow faster after confluency and form colonies in the agarose medium. All cell clones lacked adipocyte morphologies and were indistinguishable until they became confluent. To study the effect of nuclear oncogene products on preadipocyte differentiation, three clones from cells expressing each gene product were seeded at 5×10^5 cells per 60 mm dish in DMEM containing 10% fetal calf serum. 6 days after confluency (day 6; we designated the day when the cell cultures became confluent day 0), all Ad12 E1A-expressing cells began to have lipid droplets within the cells and acquire typical adipocyte morphologies. This was more prominent on day 14 (fig.2G–I). On the other hand, there were no such signs in SV40 (fig.2A–C) and polyoma virus (fig.2D–F) large T antigen-expressing clones, pSV2neo transfected 3T3-L1 cells (fig.2J,K) and the control 3T3-L1 cells (fig.2L). These results suggest that Ad12 E1A products, but not SV40 and polyoma large T antigen, enhance adipogenic differentiation under no-inducer conditions.

The representative cell clones were treated with the inducer containing dexamethasone, 1-methyl-3-isobutylxanthine and insulin for 24 h on day 7 and continued to be cultured in the regular medium lacking inducers. On day 11, all cell clones were fixed and stained with oil red O (fig.3). Clone 3–6 cells again showed a typical adipocyte morphology (fig.3, panel 1), while control 3T3-L1 (panel 3) showed only a partial appearance of lipid droplets in the cells on day 11. On day 25, lipid droplets were much more discernible in 3T3-L1 cells. In contrast, clones expressing SV40 and

Fig.2. Photographs of 3T3-L1 clones expressing nuclear oncogene products after becoming confluent. SV40 large T antigen-expressing clones (A–C), polyoma large T antigen-expressing clones (D–F), Ad12 E1A-expressing clones (G–I), 3T3-L1 cells transfected with pSV2neo (J,K) and 3T3-L1 cells (L) were cultured without treatment with inducers. Cell cultures became confluent on day 0. The picture was taken on day 14 at a total magnification of × 85.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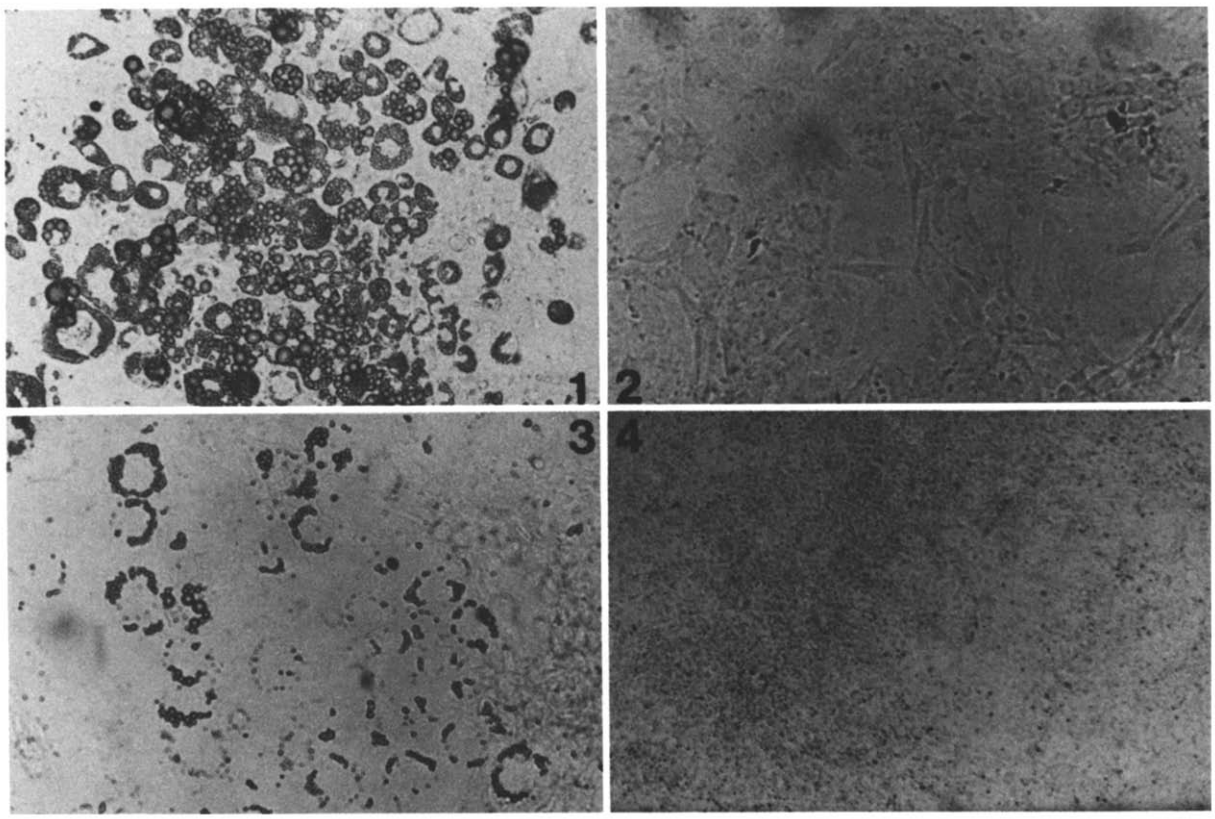

Fig.3. Photographs of clones after treatment with adipogenic inducers. The cultures of clones 3–6 (1), 2–4 (2), 1–11 (4) and 3T3-L1 cells (3) became confluent on day 0. The adipogenic inducers (0.5 mM 1-methyl-3-isobutylxanthine, 0.25 μM dexamethasone, and 1 μg/ml of insulin) were added to the cultures on day 7. Following 48 h of treatment, cells were refed with fresh medium lacking inducers. Pictures were taken on day 11 at a total magnification of $\times$ 185.

polyoma large T antigens showed no sign at all of the accumulation of lipid droplets (panels 2,4). The same result was obtained after cultivation for more than 25 days (day 25), suggesting that SV40 and polyoma large T antigen suppress adipogenic differentiation.

4. DISCUSSION

DNA tumor virus nuclear oncogene products used in this study can modulate the gene expression patterns of host cells [1]. In our study, only Ad12 E1A products could promote preadipocyte differentiation; SV40 or polyoma large T antigen would rather inhibit the differentiation, indicating that the mode of modulation differs in the two groups. A similar suppression of polyoma large T antigen was reported by Cherington et al. [11].

Recently, a number of studies have examined the role of oncogenes in tissue culture differentiation systems. For example, the activated *ras* gene has been shown to induce the rat phaeochromocytoma cell line PC12 to differentiate to a neural cell type [12]. In contrast, high-level expression of the c-*myc* gene has been shown to block the DMSO-induced differentiation of mouse erythroleukemia cells [13]. Since c-*myc* gene product is also localized in nuclei, it is interesting to examine how the overexpression of c-*myc* gene affects preadipocyte differentiation. Our preliminary result showed that the expression of mouse c-*myc* gene in 3T3-L1 cells was suppressed during the process of differentiation (unpublished), suggesting that the situation might be similar to the erythroleukemia cell system.

Ignotz and Massague [14] reported that type

transforming growth factor β (TGF-β) potentially inhibits adipogenic conversion of 3T3-L1 cells before they become committed to differentiation. The commitment point coincides with the time point immediately preceding the onset of coordinate expression of differentiation-specific proteins in 3T3-L1 cells [14]. The effective concentration (1.0 ng/ml) of TGF-β for E1A-expressing clones is 4-times higher than that (0.25 ng/ml) for the control 3T3-L1 cells (unpublished), suggesting that the competitive factors with TGF-β, which might be necessary for the induction of differentiation, seem to be specifically expressed in E1A-expressing cells. The specific genes modulated by Ad12 E1A products will be identified by characterization of cDNA libraries made from 3T3-L1 cells before and after introduction of an Ad12 E1A gene.

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