RAPID COMMUNICATION Jpn. J. Cancer Res. 81, 216–219, March 1990

Modulation of c-*myc* Expression by Transforming Growth Factor β1 in Human Hepatoma Cell Lines

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The effects of transforming growth factor β1 (TGF-β1) on cell proliferation of human hepatoma cell lines, PLC/PRF/5 and Mahlavu, were investigated under serum-free conditions. DNA synthesis was strongly inhibited in the PLC/PRF/5 cells by addition of TGF-β1 (0.5 to 4.0 ng/ml), but remained unchanged in the Mahlavu cells. Also the expression of c-*myc* mRNA was suppressed by the addition of TGF-β1 in the PLC/PRF/5 cells but not in the Mahlavu cells. These results indicate that TGF-β1 might regulate cell growth, in part, by modulating c-*myc* expression, although there is no direct proof that c-*myc* expression is really relevant to DNA synthesis mediated by TGF-β1.

Key words: Transforming growth factor β1 — c-*myc* — PLC/PRF/5 — Mahlavu

Transforming growth factor β1 (TGF-β1) is a biologically multifunctional polypeptide having a disulfide-linked dimeric structure with a molecular weight of 25 kDa.[1-3] The effect of TGF-β1 on cell growth greatly depends on the target cell type and the coexistence of other growth factors.[4-6] In general, TGF-β1 is a potent cell growth inhibitor in epithelial-type cells.[7,8] Cellular proliferation is thought to be controlled by positive and negative cell growth regulation, and TGF-β1 is a probable candidate for a negative cell growth regulator. Any disturbance of negative cell growth regulation by TGF-β1 may help to explain the active proliferation of malignant transformed cells. It is important for elucidating the role of TGF-β1 in cell growth regulation to understand the mechanisms of normal cell growth and malignant autologous proliferation.

Proto-oncogenes are normal cellular genes which encode proteins thought to play important roles in the molecular mechanism(s) of cell growth.[9-11] c-*myc* gene is a well studied proto-oncogene whose expression has been found in regenerating hepatocytes after hepatectomy[12] and in many malignant transformed cells including hepatoma cell lines.[13,14] Recent studies indicate that the nuclear protein encoded by c-*myc* gene promotes the initiation of DNA replication.[15]

Thus, it is of interest to investigate how TGF-β1 modulates the c-*myc* expression. Several studies have demonstrated the inhibition of rat hepatocyte DNA synthesis by TGF-β1,[16,17] but little is known about the effects of TGF-β1 on malignant liver epithelial cells.[18,19]

We report in this paper the effect of TGF-β1 on c-*myc* expression in two human hepatoma cell lines, PLC/PRF/5[20] and Mahlavu[21] provided by Dr. David Brown (Academic Department of Medicine, Royal Free Hospital, London); the former was dorived from a patient who was positive for hepatitis B antigen and α-fetoprotein, while the latter was from a patient negative for these markers.

PLC/PRF/5 cells and Mahlavu cells were maintained in Eagle's minimum essential medium (MEM) with 10% fetal calf serum in an atmosphere of air+5% CO_2 at 37°C. A serum-free system is desirable to avoid the influence of unknown fectors in the serum, and we employed Cosmedium (<5 mg/liter, bovine insulin, Cosmo Bio Co., Tokyo) to study DNA synthesis, because serum-free MEM was not sufficient to maintain cell growth for a long period, especially with Mahlavu cells.

For the study of DNA synthesis, cells were cultured in 96-well microplates with serum-free Cosmedium containing 0, 0.5, 1, 2, 4 ng/ml of TGF-β1 (Takara Biochemicals, Tokyo). After 46 h, [^{3}H]thymidine (1 μCi/well) was added with or without aphidicolin (10 μg/ml). Two hours later, DNA synthesis was assayed by measuring the incorporation of [^{3}H]thymidine.

To investigate the effect of TGF-β1 on c-*myc* expression, confluent dishes were washed once with MEM, then the medium was changed to serum-free MEM with or without TGF-β1 (4 ng/ml). After 3 h, the cells were harvested and total RNA was isolated according to the guanidine isothiocyanate/cesium chloride procedure.[22] For Northern blot analysis, 20 μg of total RNA was electrophoresed on 1% agarose gel and transferred to a

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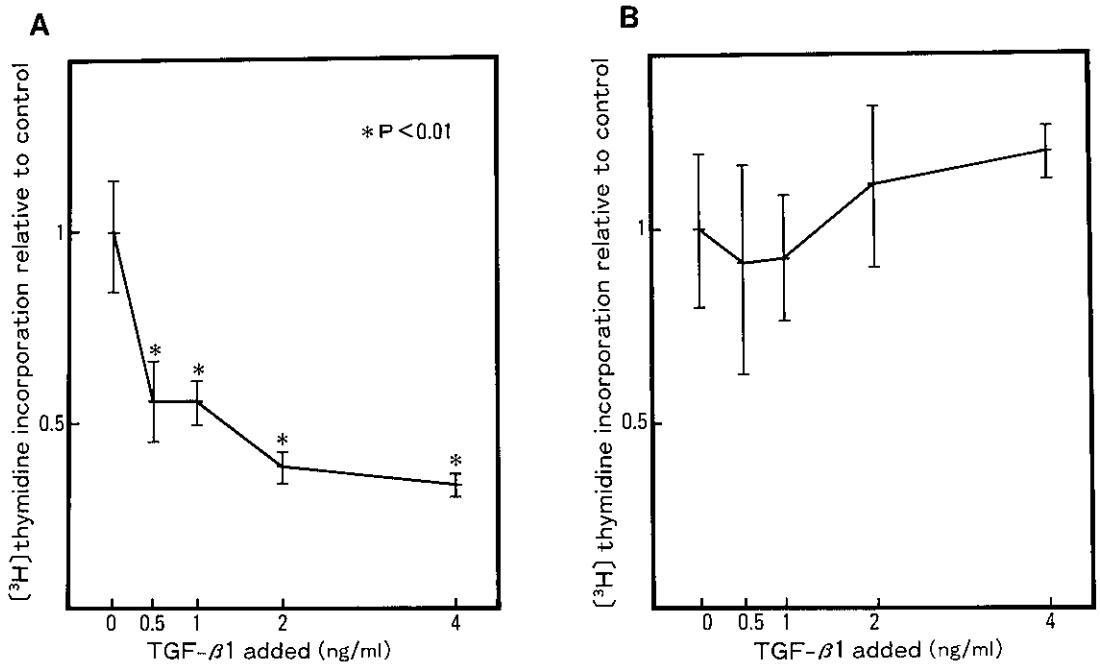
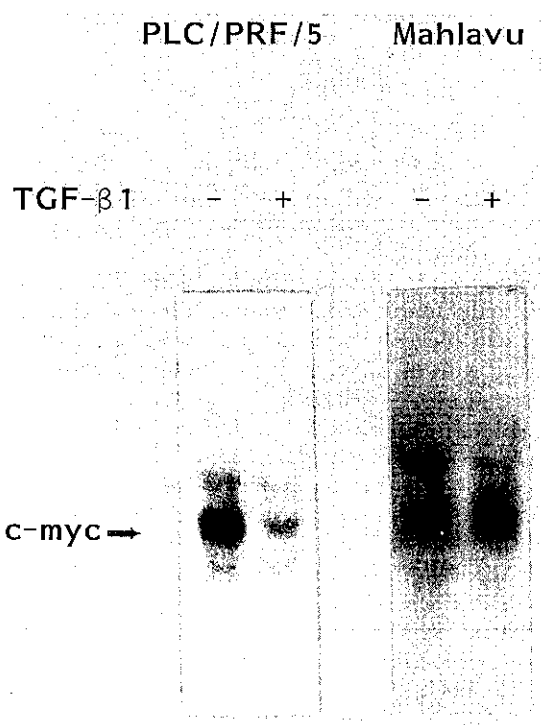


Fig. 1. Effects of TGF-β1 on [^{3}H]thymidine incorporation in PLC/PRF/5 cells (A) and Mahlavu cells (B) cultured in serum-free Cosmedium. The longitudinal axis gives the incorporation relative to the control (without addition of TGF-β1). The values represent mean$\pm$SD (n=6). Statistical analysis was done by one-way analysis of variance.



nylon membrane. Blots were hybridized using c-*myc* probe (9.0 kb)[23] labeled with [^{32}P]CTP by a Multiprime Labeling Kit (Amersham). The membrane was rehybridized with β-actin c-DNA[24] as an internal standard.

The effects of TGF-β1 on DNA synthesis in both cell lines are shown in Fig. 1. TGF-β1 strongly inhibited [^{3}H]thymidine incorporation into DNA in PLC/PRC/5 cells. Almost 50% inhibition was obtained at 0.5 ng/ml, and the [^{3}H]thymidine uptake was about 35% of the control (without addition of TGF-β1) at the maximum dose of 4 ng/ml. On the other hand, [^{3}H]thymidine uptake was not affected by TGF-β1 even at the maximum concentration in Mahlavu cells.

The effect of TGF-β1 was tested on c-*myc* expression, which is thought to be closely linked to DNA replication (Fig. 2). The Northern blot showed c-*myc* mRNA at the position corresponding to a length of 2.2 kb, and hybrid-

Fig. 2. Modulation of c-*myc* expression by TGF-β1 in PLC/PRF/5 and Mahlavu cells. Each of the cell lines was incubated with or without TGF-β1 (4 ng/ml) for 3 h in serum-free MEM. Isolated total RNA was electrophoresed (20 μg) and transferred to a nylon membrane. Blots were hybridized with c-*myc* probe labeled with ^{32}P.

ization with β-actin probe showed a similar amount of mRNA in each sample to be electrophoresed. When cultured with MEM alone, c-*myc* overexpression was observed in both cell lines. However, incubation with TGF-β1 for 3 h caused suppression of c-*myc* expression to almost 10% of the control (determined by densitometry) in the PLC/PRF/5 cells, whereas no inhibition was observed in the Mahlavu cells (data not shown).

Cellular proliferation is controlled by positive and negative cell growth regulation, and TGF-β1 is a probable candidate for negative regulation of cell growth in epithelial-type cells. Recent studies demonstrate that TGF-β1 mRNA is overexpressed in many malignant cells exhibiting active proliferation.[25] This overexpression of TGF-β1 mRNA suggests that escape from negative cell growth regulation by TGF-β1 might be attributed to the autologous proliferation of malignant cells. Cell growth is suppressed by the active form of TGF-β1 in most malignant cell lines hitherto described[26] but not in a few cell lines.[27, 28] We studied the effect of the active form of TGF-β1 on DNA synthesis in two human hepatoma cell lines, PLC/PRF/5 and Mahlavu. A differential effect of TGF-β1 was found between the two cell lines: DNA synthesis was suppressed in the PLC/PRF/5 cells but not in the Mahlavu cells.

To date, very little is known about the molecular mechanism(s) of suppression of cell growth. Information on the molecular mechanism(s) of TGF-β1 may be accessible through comparison of the alterations of molecular events between the two hepatoma cell lines. The expression of the c-*myc* proto-oncogene seems to be a molecular event closely linked to DNA synthesis, since the encoded protein has been shown to promote DNA replication.[15] The modulation of c-*myc* expression by TGF-β1 has been reported in a human breast carcinoma cell line[29] and a human colon carcinoma cell line.[30] However, these observations were carried out under the condition of c-*myc* induction by epidermal growth factor (EGF) or in the presence of serum in the culture medium. Thus, to clarify the effect of TGF-β1 itself, the concomitant effects of other growth factors must be eliminated.

Under the serum-free condition without influence of other growth factors, c-*myc* expression was strongly suppressed in PLC/PRF/5 cells, whose DNA synthesis was apparently inhibited by TGF-β1. On the other hand, no change was observed in DNA synthesis or c-*myc* expression in the Mahlavu cells. Thus, these observations indicate that TGF-β1 might regulate cell growth, in part, by modulating c-*myc* expression, although there is no direct proof that c-*myc* expression is really relevant to DNA synthesis mediated by TGF-β1. It may also be suggested that the suppression of this proto-oncogene is due to a direct function of TGF-β1 via its signal transduction pathway rather than a modulation of other growth factor functions. However, whether the modulation of c-*myc* expression by TGF-β1 occurs as the consequence of its specific signal transduction pathway and whether TGF-β1 can modulate the expression of other proto-oncogenes must await further investigation.

(Received November 8, 1989/Accepted February 6, 1990)

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