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EFFECTS OF PHORBOL ESTER ON CELL GROWTH INHIBITION BY TRANSFORMING GROWTH FACTOR β1 IN HUMAN HEPATOMA CELL LINES

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Summary The effects of phorbol ester on cell growth inhibition by transforming growth factor β1 (TGF-β1) in human hepatoma cell lines, Mahlavu and PLC/PRF/5, were investigated. TGF-β1 (2.5 to 10 pM) alone could not inhibit the growth of Mahlavu cells, whereas in the presence of 12-O-tetradecanoyl phorbol 13-acetate (TPA) at 1 ng/ml, TGF-β1 could suppress their growth in a dose-dependent manner. The growth of PLC/PRF/5 cells could be inhibited by addition of TGF-β1 (2.5 to 10 pM) alone in a dose-dependent manner, and this action was not affected by TPA (1 ng/ml). The TGF-β1 inhibition induced by TPA in Mahlavu cells could not be cancelled by addition of protein kinase C inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7) (10 μM) or staurosporin (1 nM). Thus, TPA could induce TGF-β1 inhibition of cell growth in Mahlavu cells which did not respond to TGF-β1 alone, and activation of protein kinase C does not seem to be behind this TPA action.

Transforming growth factor β (TGF-β) is a biologically multifunctional polypeptide having a disulfide-linked dimeric structure with a molecular weight of 2.5 kDa (1-3). In general, TGF-β is a potent cell growth inhibitor in epithelial cells (4,5) and is thought to be a likely candidate for a negative cell growth regulator. However, very little is known about the mechanisms of growth regulation by TGF-β.

We reported elsewhere a differential effect of TGF-β1 on the cell growth of two human hepatoma cell lines, PLC/PRF/5 (6) and Mahlavu (7). TGF-β1 strongly inhibited the growth of PLC/PRF/5 cells, but not that of Mahlavu cells. The growth inhibitory effect on PLC/PRF/5 could exert via suppression of c-myc expression (8). As further examination demonstrated that Mahlavu

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cells possess all types of TGF-β1 receptors (9-11), there may be some abnormality in the postreceptor signal transduction of TGF-β1 (12).

Phorbol ester, a potent protein kinase C (PKC) activator (13-15), potentiates the effect of TGF-β which stimulates anchor-independent growth of normal rat kidney fibroblast (NRK) cells without an increase in TGF-β1 binding (16). However, there is no evidence concerning the effects of phorbol ester on epithelial cell growth inhibition by TGF-β. In this study, we investigated the effects of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on cell growth regulation by TGF-β1 in two human hepatoma cell lines, Mahlavu and PLC/PRF/5.

Methods

Materials TGF-β1 was purchased from Takara Biochemicals, Japan, TPA was from Sigma, Staurosporin (17,18) was from Kyowa Medex Co., Japan, and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7) (19-22) was from Seikagaku Kogyo Co., Japan. Cosmedium (containing bovine insulin <5 mg/l) was purchased from Cosmo Bio Co., Japan.

Growth assay Mahlavu and PLC/PRF/5 cells were seeded at the density of $3x10^4$/well in 24-well plates. The cells were maintained with Eagle's minimum essential medium (MEM) with 10% fetal calf serum (FCS) for 24 h, then washed once with Eagle's MEM. The medium was then changed to serum-free Cosmedium with various combinations of TGF-β1, TPA and protein kinase C (PKC) inhibitors. Cell numbers were determined after 72 h.

DNA synthesis was estimated by the incorporation of [^{3}H] thymidine into DNA. Following 24 h incubation in the serum-free Cosmedium with various combinations of TGF-β1 and TPA, the incorporation of [^{3}H] thymidine (37.5 kBq/well) was counted with or without aphidichorin (10μg/ml) after six hours.

Results

Effect of TPA on cell growth inhibition by TGF-β1 Cell growth of both cell lines was not affected by TPA alone, up to 1 ng/ml. Cell numbers and DNA synthesis of Mahlavu cells were not suppressed by addition of TGF-β1 alone (2.5 to 40 pM) but were suppressed in a dose-dependent manner (2.5 to 40 pM) in the presence of TPA (1 ng/ml) (Fig. 1A, Fig. 2A). In PLC/PRF/5 cells, cell numbers and DNA synthesis were suppressed by addition of TGF-β1 alone in a dose-dependent manner (2.5 to 40 pM). This inhibitory action of TGF-β1 was not affected by addition of TPA (1 ng/ml) (Fig. 1B, Fig.2B). Similar results were obtained when Eagle's MEM with 10% FCS was used instead of serum-free Cosmedium.

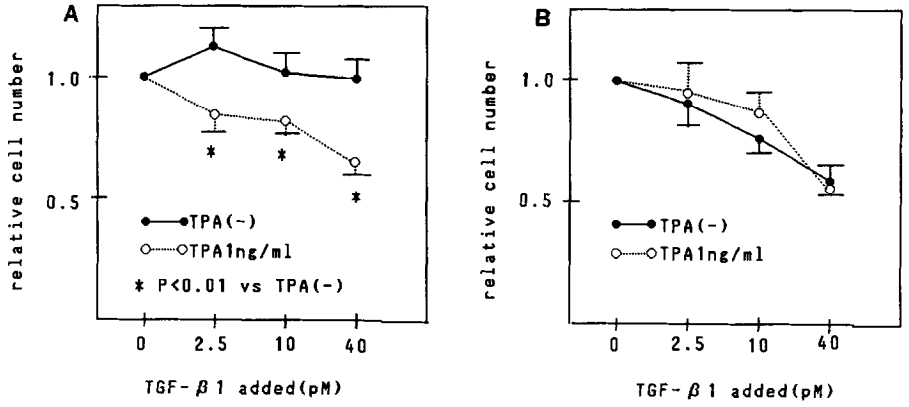

Fig. 1. Effects of TPA on cell growth regulation by TGF-β1 in Mahlavu (A) and PLC/PRF/5 cells (B) cultured in serum-free Cosmedium. The longitudial axis gives the cell number relative to the control (without addition of TGF-β1). The values represent mean ± S.D. (n=3). Statistical analysis was done by one-way analysis of variance.

Effects of H7 and staurosporin on TPA-induced cell growth inhibition by TGF-β1 in Mahlavu cells The cell growth inhibition by TGF-β1 (40 pM) in the presence of TPA (0.5 ng/ml) was not affected by addition of PKC inhibitors, H7 (10 μM) or staurosporin (1 nM)(Fig. 3).

Discussion

TGF-β1 is a potent biological regulator of cell growth and differentiation. We investigated effects of TPA on the growth response of Mahlavu and

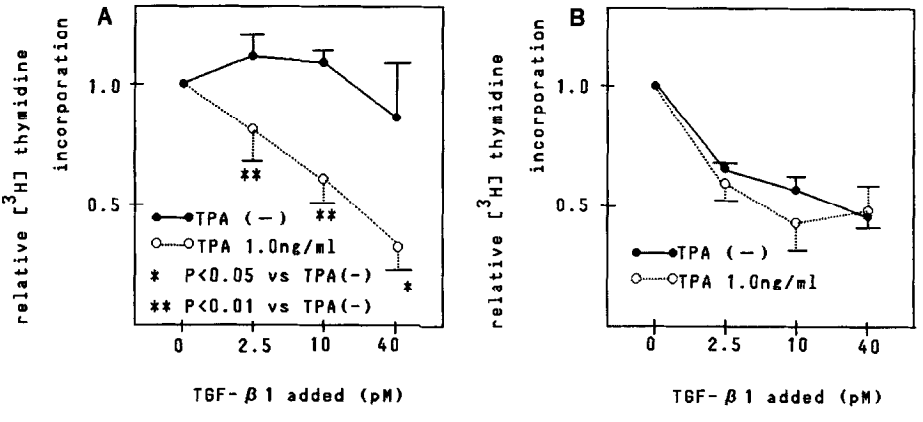

Fig. 2. Effects of TPA on DNA synthesis regulation by TGF-β1 in Mahlavu (A) and PLC/PRF/5 cells(B) cultured in serum-free Cosmedium. The longitudial axis gives the incorporated [^{3}H]thymidine relative to the control (without addition of TGF-β1). The values represent mean ± S.D. (n=4). Statistical analysis was done by one-way analysis of variance.

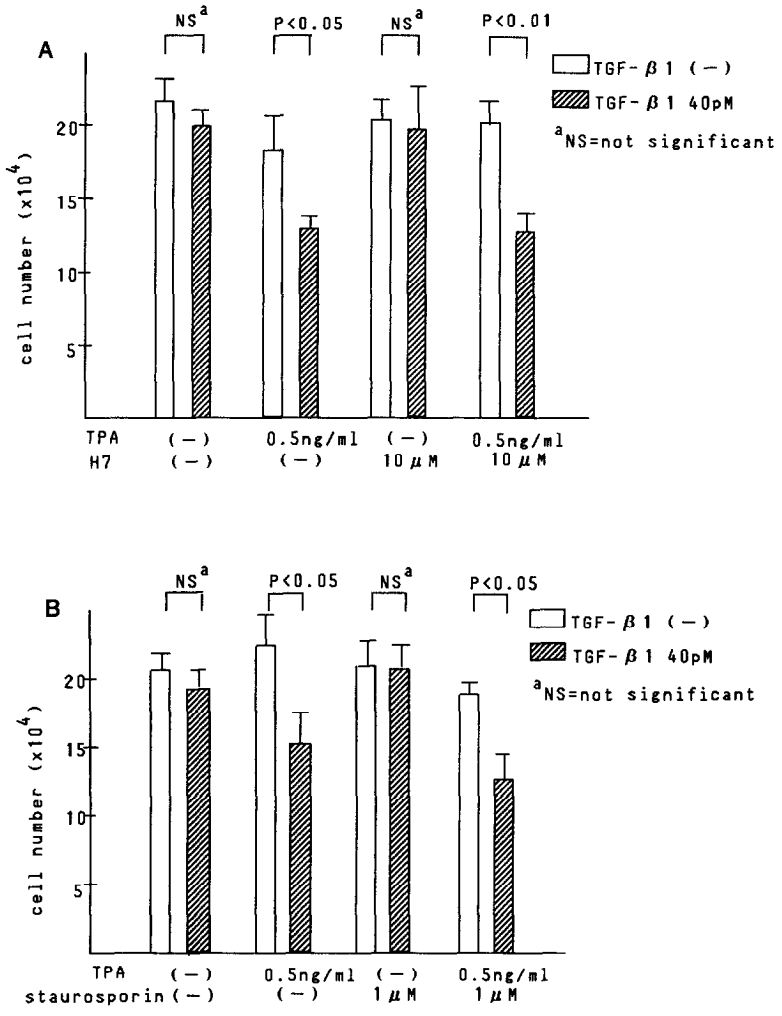

Fig. 3. Effects of PKC inhibitors, H7 (A) and staurosporin (B), on cell growth inhibition by TGF-β1 induced by TPA in Mahlavu cells. The values represent mean ± S.D. (n=3). Statistical analysis was done using Student's T test.

PLC/PRF/5 cells to TGF-β1, and found that TPA was needed to modulate the growth response of Mahlavu cells to TGF-β1, but not that of PLC/PRF/5 cells. As all types of TGF-β1 receptors are present in Mahlavu cells, their unresponsiveness to TGF-β1 may be due to some abnormality in the postreceptor signal transduction of TGF-β1 (12). PLC/PRF/5 cells, on the other hand, possess all types of TGF-β1 receptors and can respond to the active form of TGF-β1, indicating no impairment in postreceptor signal transduction of TGF-β1 (12). In Mahlavu cells, TPA may be needed to remove the impairment in the TGF-β1 signal transduction in order to enable TGF-β1 to exert its action.

TPA, the most active derivative among the phorbol diester tumor promotors, has been reported to affect a large number of biological systems (23-25). Most of the effects of TPA are mediated by activation of PKC (13-15). Exogenous addition of TPA causes translocation of PKC from the cytosol to the membrane, and various proteins are phospholylated by PKC (26,27). In order to clarify whether or not the induction of the TGF-β1 action by TPA is mediated through the activation of PKC, we investigated the effects of further addition of PKC inhibitor, H7 and staurosporin, which are considered to inhibit PKC through different mechanisms (17-22). The effects of TPA on inducing cell growth inhibition by TGF-β1 were not cancelled by the addition of H7 or staurosporin. These results suggest that the induction of the inhibitory effect of TGF-β1 by TPA in Mahlavu cells is not due to the activation of PKC. Several lines of evidence have been reported indicating that some biological actions of TPA do not involve activation of PKC (28-32). However, detail mechanisms of the inhibitory effect of TGF-β1 induced by TPA in Mahlavu cells remain to be clarified.

In summary, we demonstrated that TPA can induce cell growth inhibition by TGF-β1 in Mahlavu cells, which do not respond to TGF-β1 alone. This induction does not seem to be due to the activation of PKC.

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