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EXPRESSION OF A NEW TYPE HIGH MOLECULAR WEIGHT RECEPTOR (TYPE V RECEPTOR) OF TRANSFORMING GROWTH FACTOR β IN NORMAL AND TRANSFORMED CELLS

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Summary — A new type high molecular weight receptor (type V receptor) of transforming growth factor β (TGF-β) was recently purified from bovine liver plasma membranes and appears not to be related to receptors previously described for TGF-β (Pauline O'Grady, Ming-Der Kuo, Joseph J. Baldassare, Shuan Shian Huang and Jung San Huang [1991] *J. Biol. Chem.* **266**:8583-8589). This type V receptor may be important in the regulation of cell growth by TGF-β. We examined its distribution in a wide range of normal and transformed cells. The type V receptor was found to be expressed in many normal cells including cells of epithelial, endothelial, fibroblastic and chondrocytic origins. However, a number of human epithelial tumor cells (5 out of 6 examined) did not express detectable levels of the type V TGF-β receptor. These results suggest that loss of the type V receptor may potentially contribute to the transformed state of certain epithelial tumor cells.

Transforming growth factor beta (TGF-β) is a family of structurally homologous, dimeric proteins (TGF-β_1, TGF-β_2 and TGF-β_3) which displays multiple biological activities including the negative and positive regulations of cell growth, the regulation of extracellular matrix protein synthesis, angiogenesis and the differentiation of several cell lineages (1-3). Such activities imply a physiological role for TGF-β in wound repair (4), tissue fibrosis (5) and morphogenesis (1-3).

TGF-β binds to specific cell surface receptors which appear to mediate its biological activities (6). A number of differentially sized receptors for TGF-β have been identified on almost all cultured cells namely type I (M_r ~ 53,000), type II (M_r ~ 83,000-110,000), and type III (M_r ~ 280,000-310,000) (7). The type IV receptor (M_r ~ 60,000) has only been identified in

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pituitary cells (8). More recently, a new type high molecular weight receptor (type V receptor) of TGF-β has been identified by our laboratory (M_r ~ 400,000) and has been purified from bovine liver membranes (9). The exact role of the different TGF-β receptors is unclear.

The types I, II and III TGF-β receptors co-express on most cell lines studied, although the relative levels of the different receptors may vary according to cell type (10,11). Such a widespread distribution suggests an important role for all three receptors in TGF-β actions. Cells lacking the type III receptor still respond to TGF-β with growth inhibition (11) and genetic evidence further implicates the types I and II receptors in mediation of the growth inhibitory response (12). However, it has also been shown that TGF-β_1 and TGF-β_2 exhibit similar potencies in terms of growth inhibition and only the type III receptor can bind TGF-β_1 and TGF-β_2 with identical affinities (13). It is thus possible that the type III receptor, when present, can modulate the function of the types I and II receptors.

The contribution of the recently identified type V TGF-β receptor in mediation of TGF-β actions has not yet been studied. Its presence in a tissue such as bovine liver suggests its physiological relevance. It has also been found to be co-expressed in cultured mink lung epithelial cells together with the types I, II and III TGF-β receptors (9).

A study of its distribution in many different cell types should help to elucidate its role in TGF-β function. In this communication, we examined the expression of the type V TGF-β receptor in a wide range of cultured cells, both normal and neoplastic. Like the types I, II and III TGF-β receptors, the type V TGF-β receptor showed a wide distribution in cultured normal cells. Interestingly, the type V TGF-β receptor was undetectable in five of the six epithelial tumor cells studied.

Materials and Methods

Materials — $Na^{125}I$, TGF-β_1, TGF-β_2, mouse epidermal growth factor, recombinant human basic fibroblast growth factor, recombinant human platelet-derived growth factor (c-sis), high molecular mass protein standards, chloramine T and disuccinimidyl suberate (DSS) were obtained as previously described (9). Bovine insulin was obtained from Gibco. Mink lung epithelial cells (Mvl.Lu), normal rat kidney cells (NRK), baby hamster kidney cells (BHK), rat pheochromocytoma cells (PC-12), African green monkey kidney cells (BSC-1), and mouse embryonic fibroblast (NIH 3T3) were maintained as previously described (19). Fetal bovine heart endothelial cells (FBHE), human

mammary carcinomas (MCF-7 and SK-BR-3), human choriocarcinoma cells (BEWO), human hepatoma cells (HepG2) and human epidermoid carcinoma were obtained from The American Type Culture Collection. Human osteoblasts (OST) were provided by Dr. C.C. Tsai, Department of Pathology, St. Louis University School of Medicine. Rat chondrocytes (CHON) and human hepatocarcinoma cells (Hep) were provided by Dr. W.J. Yang, Department of Medicine, Washington University School of Medicine, St. Louis, MO. Simian sarcoma virus (SSV) - transformed NRK and NIH 3T3 cells were provided by Drs. S.A. Aaronson and K.C. Robbins (National Cancer Institute). SSV-transformed NP1 cells were provided by Dr. Flossie Wang-Staal (National Cancer Institute) and human umbilical vein endothelial cells (HUVEC) were provided by Dr. J. Olander, Department of Biochemistry and Cell Culture, Monsanto Company, St. Louis, MO. All cells were grown at 37°C in a humidified 5% CO_2 atmosphere. MCF-7, SK-BR-3, BEWO and HepG2 cells were grown in RPMI-1640 medium containing 10% fetal calf serum. All other cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Endothelial cell cultures were supplemented with 5 ng/ml aFGF.

Affinity labeling of TGF-β receptors in cultured cells — All cells were grown to confluence in 35-mm Petri dishes. Affinity labeling of TGF-β receptors with ^{125}I-TGF-β (TGF-β_1) in the presence of a bifunctional reagent, disuccinimidyl suberate (DSS) and subsequent electrophoresis and autoradiography was carried out as previously described (9).

Results and Discussion

Ligand specificity of the type V TGF-β receptor for TGF-β.

The TGF-β receptors (types I, II and III) have been shown to bind TGF-β_2 as well as TGF-β_1, but not platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), or epidermal growth factor (EGF) (1-3). The ligand specificity of the type V TGF-β receptor was therefore examined. Mink lung epithelial cells were incubated with ^{125}I-TGF-β (^{125}I-TGF-β_1) in the presence and absence of a 100-fold excess of unlabeled TGF-β_2 or other growth factors (PDGF, EGF, bFGF and insulin). The ^{125}I-TGF-β receptor complexes were then analyzed by cross-linking with DSS followed by 5.5% SDS-polyacrylamide gel electrophoresis and autoradiography. As can be seen in Fig. 1, a 100-fold excess of unlabeled TGF-β_2 blocked the formation of the type V receptor ^{125}I-TGF-β complex as well as the other receptor type-^{125}I-TGF-β complexes (Fig. 1A). This complex formation was not significantly affected in the presence of a 100-fold excess of EGF, bFGF, PDGF or insulin (Fig. 1B). These results suggest that, like other types of TGF-β receptors (types I, II and III), the type V TGF-β receptor is specific for TGF-β_1 or TGF-β_2 binding and thus may be important for their cellular functioning.

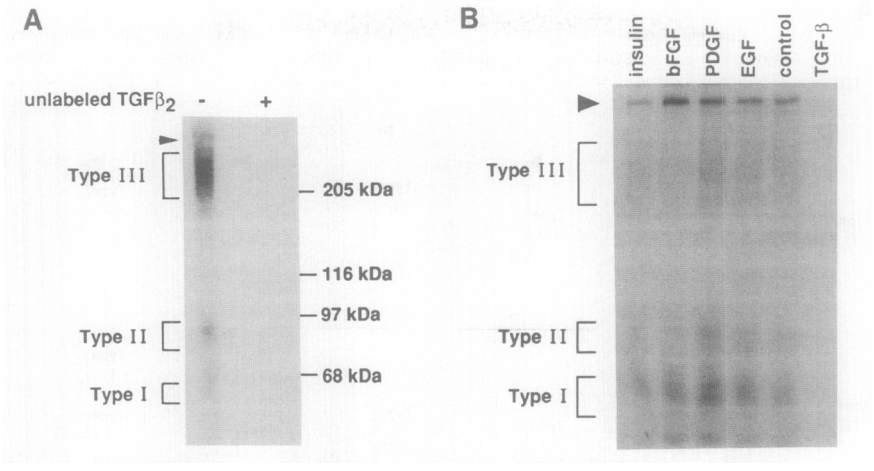

Fig.1. Ligand specificity of the type V TGF-β receptor. Confluent monolayers of mink lung epithelial cells in 35-mm Petri dishes were incubated for 3.5 h at 4°C with ^{125}I-TGF-β (40 pM) in the presence and absence of a 100-fold excess of unlabeled TGF-β_2 (A), PDGF, EGF, bFGF or insulin (B). The ^{125}I-TGF-β-affinity labeled receptor species were then analyzed on 5.5% SDS-polyacrylamide gel followed by autoradiography. The arrowhead indicates the location of the type V TGF-β receptor-^{125}I-TGF-β complex. The brackets indicate the locations of ^{125}I-TGF-β-affinity labeled types I, II and III TGF-β receptors.

Distribution of the type V TGF-β receptor in cultured normal cells.

Since the type V TGF-β receptor was found to co-express in mink lung epithelial cells with all three of the other TGF-β receptor types and was also found in African green monkey kidney cells (BSC-1) (9), the expression of the type V TGF-β receptor was examined in a wide range of cultured normal cells. Cells were incubated with ^{125}I-TGF-β, and the ^{125}I-TGF-β-TGF-β receptor complexes were then analyzed by cross-linking with DSS followed by 5.5% SDS-polyacrylamide gel electrophoresis and autoradiography. As shown in Fig. 2A and Table I, the type V TGF-β receptor was detected in many normal cells including epithelial cells (Mvl.Lu, BSC-1) as previously shown (9), fibroblasts (BHK, NIH 3T3, NRK), endothelial cells (FBHE) and in chondrocytes. These cells were derived from a number of mammalian species including human, monkey, cow, hamster, mink, mouse and rat. The distribution of the type V TGF-β receptor is thus widespread as shown for the other three TGF-β receptor types. Since there is no cell line which exhibits exclusively the type V TGF-β receptor, it is difficult to correlate the presence or absence of this receptor with a role in TGF-β functioning. However, its broad expression in different species and different cell types suggests an important role for this receptor in TGF-β actions.

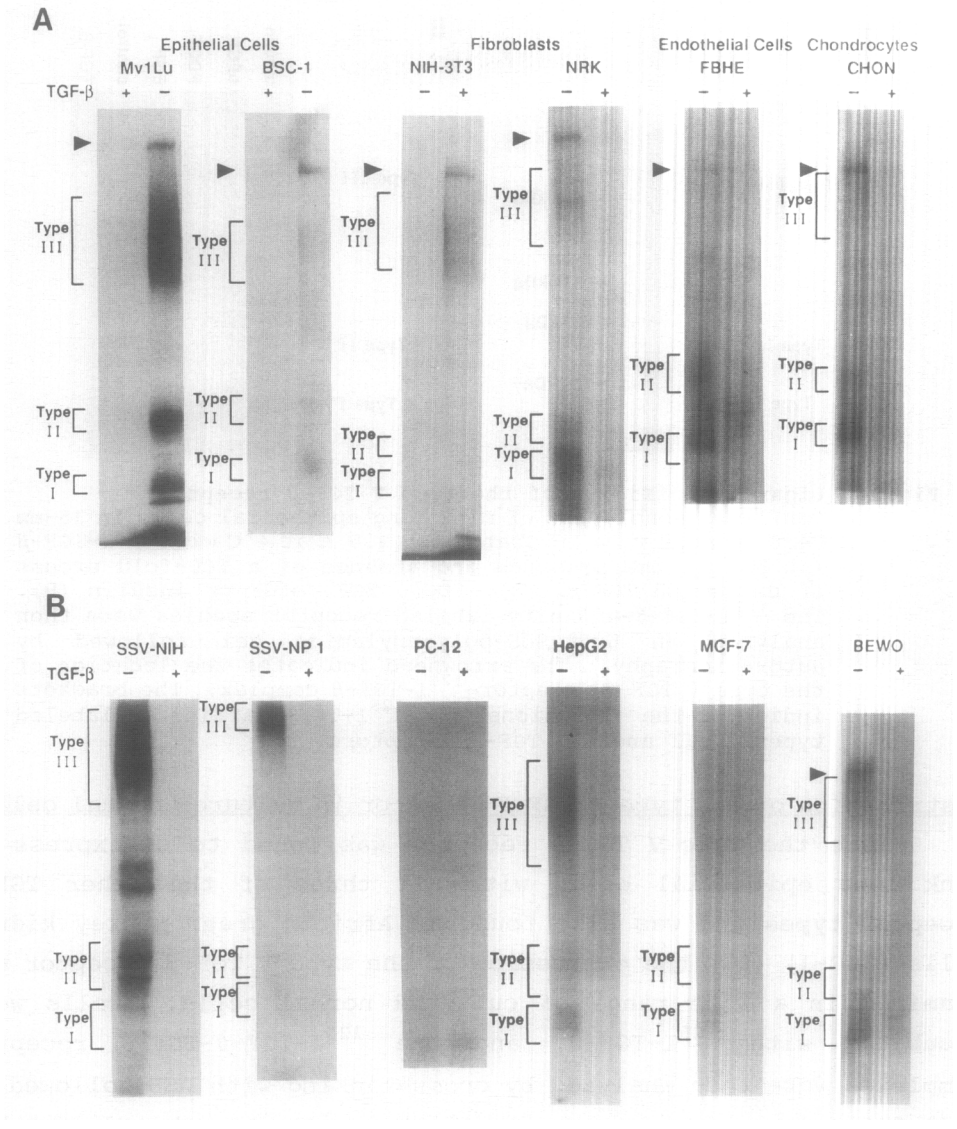

Fig. 2. Identification of ^{125}I-TGF-β receptor in cultured normal (A) and transformed cells (B).
Cells grown in 35-mm Petri dishes were incubated for 3.5 h at 4°C with ^{125}I-TGF-β (40 pM) in the presence (+) and absence (-) of a 100-fold excess of unlabeled TGF-β. The ^{125}I-TGF-β-affinity labeled TGF-β receptor species were then identified as described in the legend to Fig. 1. The arrowhead indicates the location of the type V TGF-β receptor-^{125}I-TGF-β complex. The brackets indicate the locations of ^{125}I-TGF-β-affinity labeled types I, II and III TGF-β receptors.

Distribution of the type V TGF-β receptor in cultured tumor cells.

The role of TGF-β in regulation of tumor growth is under intense investigation at present (1-3). Absence of TGF-β receptor(s) may be one of the mechanisms whereby tumors could show altered sensitivity to the actions of TGF-β. There are several

Table I. Expression of the Type V TGF-β Receptor and Other TGF-β Receptor Types in Normal and Transformed Cell Lines

Cell Type	Name	Species	TGF-β Receptor Type[a] Type V	III	II	I
Normal Cells						
Epithelial						
lung	Mv1.Lu	mink	+	+	+	+
kidney	BSC-1	monkey	+	±[b]	+	+
Fibroblast						
kidney	BHK	hamster	+	+	+	+
kidney	NRK	rat	+	+	+	+
embryo	3T3	mouse	+	+	+	+
Endothelial						
fetal-heart	FBHE	cow	+	±[b]	+	+
umbilical-vein	HUVEC	human	+	±[b]	+	++
Chondrocyte						
fetal-bone	CHON	rat	+	+	+	+
Osteoblast						
bone	Ost	human	-	+[c]	+	+
Transformed Cells						
Epithelial						
breast	MCF-7	human	-	-	+	+
breast	SK-BR-3	human	-	-	+	+
vulva	A431	human	-	+[c]	+	+
liver	HepG2	human	-	+	+	+
liver	Hep	human	-	+[c]	+	+
placenta	BEWO	human	+	+	+	+
Fibroblast						
kidney	SSV-NRK	rat	+	+	+	+
embryo	SSV-3T3	mouse	±[d]	+[c]	+	+
skin	SSV-NP1	monkey	±[d]	+[c]	+	+
Adrenal						
chromaffin	PC-12	rat	-	-	-	-

[a] The TGF-β receptors were detected by a cross-linking assay in which the TGF-β receptor-^{125}I-TGF-β complex was cross-linked by DSS and analyzed by 5.5% SDS polyacrylamide gel electrophoresis followed by autoradiography. The plus (+) indicates the presence of this type TGF-β receptor in these cells, whereas the minus (-) indicates the absence of the receptor.

[b] The level of this receptor type was too low to determine accurately.

[c] The type III receptor showed a molecular weight of >400 kDa.

[d] The type V receptor was masked by the type III receptor which showed an unusually high molecular weight (>400 kDa).

reports in the literature of cultured tumor cells which have lost the ability of their normal counterparts to respond to TGF-β with growth inhibition (14-19). However, although in some cases this loss of response appears to be correlated with a decrease in TGF-β receptor number (14,15,18), the receptor profile of most of these tumors was not reported. For these reasons, we investigated the expression of the type V TGF-β receptor in a number of cultured

tumor cells (9). As shown in Fig. 2B and Table I, the type V TGF-β receptor was not detected in most of the tumor cell lines examined even following prolonged exposure of the autoradiograph (data not shown). With the exception of the PC 12 cells, all of these tumors expressed both the type I and type II TGF-β receptors and most expressed a type III proteoglycan receptor also.

The absence of the type V TGF-β receptor in five of the six epithelial tumors studied is interesting and induces speculation on the possible role of the type V TGF-β receptor in tumor biology. Since TGF-β is the most potent growth inhibitor known to date for epithelial cells (20), it is possible that loss of the type V receptor could contribute to an escape from this growth inhibitory effect and thus to an enhancement of tumor growth. Alternatively, loss of the type V TGF-β receptor could potentially alter the cell's response to TGF-β in terms of extracellular matrix breakdown. TGF-β was recently shown to suppress the invasiveness of tumor cells *in vitro* by increasing synthesis and secretion of tissue inhibitor of metalloprotease (TIMP) (21). The loss of the type V receptor may provide a mechanism for certain epithelial tumor cells to escape from the possible TGF-β counter action on its invasiveness.

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