Communication

Requirement of Transforming Growth Factor-β (TGF-β) Type II Receptor for TGF-β-induced Proliferation and Growth Inhibition*

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Growth regulation of fibroblasts is important for lung development and repair of lung injury. In this study, we investigated the role of transforming growth factor-β (TGF-β) type II receptor in the TGF-β-dependent proliferative response of lung fibroblasts. TGF-β stimulated the proliferation of adult lung fibroblasts at a low concentration (1 ng/ml), but inhibited the growth of fetal lung fibroblasts in a dose-dependent fashion (0.1–10 ng/ml). Cross-linking and Northern analysis demonstrated that the two lung fibroblast cell lines expressed the TGF-β type I receptor (TβRI) and type II receptor (TβRII). We overexpressed in lung fibroblasts a truncated derivative of TβRII that lacked the cytoplasmic serine/threonine kinase domain (TβRIIΔK). TβRIIΔK was a dominant-negative inhibitor of TGF-β signal transduction blocking not only TGF-β-induced mitogenic action upon adult lung fibroblasts but also TGF-β-induced growth inhibition of fetal lung fibroblasts. The results indicate that the type II receptor is indispensible for mediating both the mitogenic and antiproliferative effects of TGF-β upon lung fibroblasts.

Transforming growth factor-β (TGF-β) is a family of multifunctional cytokines that regulates cell growth, differentiation, and extracellular matrix deposition (1). Three isoforms TGF-β1, -2, and -3 have been identified in lung and found to act on many different lung-derived cell types and to regulate a wide variety of cellular activities (2, 3). TGF-β family elicits their biological effects on cells through binding to cell surface transmembrane receptors. A number of different types of putative receptors for TGF-β, including three distinct size classes termed type I (TβRI, 50–60 kDa), type II (TβRII, 75–85 kDa), and type III (TβRIII, 280-kDa proteoglycan with a 120-kDa core protein), have been identified by affinity cross-linking experiments (4, 5). Molecular cloning of cDNAs coding type I and type II receptors for the TGF-β superfamily have shown that both types belong to a novel family of transmembrane serine/threonine kinases with a small extracellular domain, a single transmembrane segment, and an intracellular region with a serine/threonine kinase domain (6–8). Sequence analysis of TβRIII revealed that it is a transmembrane proteoglycan with a short and highly conserved cytoplasmic domain that has no apparent signal motif (9, 10). Current evidence indicates that a complex of TβRI and TβRII, but not the individual components, mediates TGF-β signal transduction. A ligand-induced heterodimer model was proposed for TGF-β signal transduction (11, 12).

TGF-β may act as either a positive or a negative regulator of cell division. TGF-β stimulates the proliferation of mesenchymal-derived cells such as fibroblasts and osteoblasts, but acts as a powerful growth inhibitor of cells of epithelial and endothelial origin (13, 14). TGF-β inhibits epithelial cell proliferation by delaying or arresting progression through the late portion of G1 (15). TβRII was found to be essential for TGF-β growth inhibition signal. Mv1Lu mink lung epithelial cells are highly responsive to the growth inhibition of TGF-β. A chemically mutated Mv1Lu cell line defective in TβRII lacks TGF-β-induced growth inhibition. Transfection of the human TβRII to this mutant cell line restored the inhibition of growth by TGF-β (16, 17). A similar conclusion has been drawn by expression of a kinase-defective truncation of the human TβRII in Mv1Lu mink lung epithelial cells (18).

TβRII is required to mediate antiproliferative responses to TGF-β, but its involvement in TGF-β signaling that lead to growth stimulation has not been established. In the present studies, we demonstrate a bifunctional action of TGF-β in lung fibroblast cells. An adult rat lung fibroblast cell line expressed TβRII and was responsive to the growth stimulation of TGF-β, whereas a fetal rat lung fibroblast cell line expressed TβRII and displayed an unexpected response, growth inhibition, to TGF-β. To further determine the role of TβRII in modulating the growth stimulation effects of TGF-β, we transfected into rat lung fibroblast cells an expression plasmid containing rat TβRII cDNA that was lacking the kinase domain. Overexpression of the dominant-negative TβRII mutant blocked both the stimulating and the inhibitory effects of TGF-β on rat lung fibroblasts. These experiments provide evidence for a functional role of TβRII in TGF-β-induced proliferation and growth inhibition of lung fibroblasts.

MATERIALS AND METHODS

Cell Cultures—Fetal rat lung fibroblasts were isolated from day 16 gestational age fetal rat lung tissues as described (19). Adult rat lung fibroblasts isolated from 9-week-old rats were kindly provided by Dr. J. Clarke McIntosh from Duke University Medical Center. These cells exhibited typical fibroblastoid morphology, and they were vimentin-positive and cytokeratin-negative. These lung fibroblasts were also characterized by expression of extracellular matrix protein tenascin and fibronectin (19). Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal calf serum. Cultures were grown in humidified 5% CO2 and 95% air at 37 °C.

Mitogenesis Assay—[3H]Thymidine incorporation was used to determine TGF-β sensitivity of fibroblasts to exogenous TGF-β treatment. Fetal rat lung fibroblasts or adult rat lung fibroblasts were plated in 24-well plates at a density of 2 × 104 cells/well in DMEM supplemented...
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with 10% fetal bovine serum. After 48 h of incubation, the cells were serum-starved for 24 h in serum-free medium. Quiescent cultures were then incubated with serum-free DMEM in the presence of various concentrations of TGF-β (0.1 ng/ml-10 ng/ml), as indicated. After receiving a 6-h pulse with 2 μCi/ml of [3H]thymidine (Amersham Corp.), cells were rinsed with phosphate-buffered saline three times, and twice with 10% TC100, then lysed in 10% NaOH. The amount of [3H]thymidine incorporated was analyzed by liquid scintillation counting.

Receptor Constructs—A 1762-base pair cDNA containing the full-length coding sequence for TβRII was used to optimize the transfection conditions. Under the transcriptional control of the cytomegalovirus immediate early gene promoter, was used to optimize the transfection conditions.

Northern Analysis—Total RNA was from cells prepared by the guanidine thiocyanate/cesium chloride method. PolyA+ RNAs were selected with the PolyATract® mRNA isolation kit (Promega, Madison, WI). Two μg of mRNA was fractionated on an agarose gel, transferred to Nytran nylon membrane (Schleicher and Schuell) and fixed by a StrataCross™ UV cross-linker (Stratagene, La Jolla, CA). Filters were hybridized at 42 °C in 50% formamide solution containing 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM Na2HPO4, and 1 mM EDTA), 1 μg/ml bovine serum albumin at 4°C. Bound [3H]TGF-β1 was detected by autoradiography.

RESULTS

Growth responses to TGF-β1 by rat lung fibroblasts were examined. Confluent fetal rat lung fibroblasts and adult rat lung fibroblasts were made quiescent and exposed to different concentrations of TGF-β1. The adult rat lung fibroblast cell line was responsive to the growth stimulation of TGF-β1 as expected of most fibroblast cells, whereas fetal rat lung fibroblasts displayed an unexpected response, growth inhibition (Fig. 1A). The effects of TGF-β1 on the proliferation of adult lung fibroblasts were dependent on concentration (Fig. 1A). TGF-β1 stimulated the proliferation of adult lung fibroblasts at concentration less than 5 ng/ml. Maximal effects were observed at a concentration of 1 ng/ml, and TGF-β1 had no effect at concentrations of over 5 ng/ml. In contrast, TGF-β1 inhibited the proliferation of fetal lung fibroblasts in a dose-dependent manner up to 10 ng/ml after a 20-h treatment with TGF-β1 (Fig. 1B). [3H]Thymidine incorporation peaked at 24–36 h in adult lung fibroblasts treated with TGF-β1, but inhibition of DNA synthesis of fetal lung fibroblasts by TGF-β1 persisted for up to 72 h (data not shown).

In order to elucidate a possible mechanism whereby TGF-β1 exerts its actions on fibroblast proliferation, the expression of TGF-β receptors by adult and fetal lung fibroblasts were evaluated. Cross-linking of 125I-TGF-β1 to fetal lung fibroblasts or adult lung fibroblasts revealed three species of receptors with apparent molecular masses of 60, 85 and 280 kDa (Fig. 2A). These proteins were equivalent to the TGF-β type I, II, and III receptors, respectively. A 40-kDa component was seen in fetal lung fibroblasts, but not in adult lung fibroblasts. It is not clear whether it was an isoform of the TGF-β type I receptor or an
uncharacterized TGF-β binding protein. A lower molecular mass species (35 kDa) was also detected in both adult and fetal lung fibroblasts.

The expression of TGF-β type I and II receptor were further examined by Northern blot analysis (Fig. 2B). TpRII mRNA was detected as a 5.1-kilobase species expressed in adult lung fibroblasts and in fetal lung fibroblasts. Two TpRI mRNA species of approximately 6.1 and 4.0 kilobases were observed in adult lung fibroblasts and in fetal lung fibroblasts. TpRII and TpRI were differentially expressed in fetal and adult lung fibroblasts.

To examine the significance of type II receptor in mediating the growth-promoting and growth inhibition effects of TGF-β, we used a dominant-negative inhibitory approach to create a kinase-deficient cytoplasmic deletion mutant of rat TpRII (TpRIIΔK). The truncated TpRII and wild type TpRII were cloned into pCDNA3, under the transcriptional control of the cytomegalovirus immediate early gene promoter and enhancer. We transfected rat lung fibroblasts with TpRIIΔK or TpRII. The expression level of the truncated receptor was tested by affinity labeling of transfected cells with 125I-TGF-β1 (Fig. 3). Fibroblasts transfected with TpRIIΔK yielded a TGF-β affinity-labeled product of 45 kDa, the predicted size of the truncated receptor TpRII. A high level of TpRIIΔK were expressed on the cell surface of fetal and adult lung fibroblasts. The truncated receptor was able to bind ligand. 125I-TGF-β1 binding to TpRIIΔK was efficiently competed by unlabeled ligand, the same as the wild type TpRII binding.

125I-thymidine incorporation was measured to assess the proliferation rate of lung fibroblasts expressing the truncated TpRII. Adult lung fibroblasts were transfected with TpRIIΔK or empty vector. Transfected fibroblasts were cultured in the absence or presence of TGF-β1 (1 ng/ml) for 20 h. DNA synthesis was assayed by measuring [3H]thymidine incorporation; B, restoration of TGF-β1-dependent growth response. Adult lung fibroblasts were transfected with 1 μg of TpRIIΔK and 1 μg of wild type rat TpRII. The cells were treated with TGF-β1 for 20 h and then assayed for DNA synthesis. The bars represent the means ± S.E. (n = 3).

DISCUSSION

Proliferation of fibroblasts is an important aspect of lung development and is also a key feature of repair of lung injury. Expression of TGF-β isoforms has been detected in the lung at critical times during development (2, 23). The expression pattern and known biological activities of TGF-β suggest an important role for this factor in lung development. In this study, the effects of TGF-β on growth of fetal and adult lung fibroblasts were characterized, and the role of TpRII in TGF-β-induced signaling was examined.
Our data show that TGF-β stimulates proliferation of adult fibroblasts at low concentration but remains inactive at higher concentration, while inhibiting growth of fetal fibroblasts regardless of concentration. An example of the bifunctional nature of TGF-β has been reported in human smooth muscle cells (24). TGF-β is mitogenic only when used at lower concentration, whereas at higher concentration it inhibits DNA synthesis. More recent studies have demonstrated that TGF-β can act as a negative or positive growth regulator in two sublines of the same epithelial cells, depending on their commitment to differentiation (25). TGF-β activates two different signal transduction pathways through activating different Ras proteins and myelin basic protein kinases (26).

We examined the possible relationship between the bifunctional action of TGF-β and the expression of its cellular receptors. The two fibroblast cell lines possess all three TGF-β receptors; however fetal fibroblasts expressed a much higher level of cell surface type II receptor at the mRNA and the protein level than adult lung fibroblasts. The finding is in agreement with our previous study (20) that the expression of TβRII is developmentally regulated in the lung. This raises the possibility that the bifunctional action of TGF-β on lung fibroblasts may depend on their developmental stage.

We show that the kinase-deleted truncation of TβRII acts as a dominant inhibitor of TGF-β signal transduction. The proliferative and the growth inhibition of TGF-β were abolished by overexpression of TβRIIΔK. TGF-β-induced signaling events leading to growth stimulation are likely to be different at some level from that of growth inhibition. TGF-β increases c-fos expression in growth-inhibited cells but not in growth-stimulated cells (27). An increase in retinoblastoma protein phosphorylation is seen in TGF-β-induced proliferation (28). However, our results indicated TβRII is essential for both pathways. This finding suggests that binding of TGF-β to TβRII may be a common step required for the TGF-β signaling cascade. The TGF-β signal transduction pathways for the growth stimulation and the growth inhibition may diverge after binding of TGF-β to TβRII and then different intracellular signals might be activated. An intriguing question raised by this study is how TGF-β exerts its multiplicity of effects through interaction with its transmembrane receptors. The signaling specificity could occur through interaction of TβRII with different types of type I receptor. The characterization of other members of TGF-β receptor family, particularly type I receptors, might allow us to determine the mechanism of multiple effects of TGF-β.

In conclusion, we found that TGF-β stimulates proliferation of adult lung fibroblasts, while inhibiting growth of fetal lung fibroblasts. We have shown that the truncated form of TβRII blocked both growth-stimulating and inhibition effects of TGF-β. The results suggest that TGF-β type II receptor is indispensable for the signal transduction pathway and the diverse regulatory effects of TGF-β.

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