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Effects of Transforming Growth Factor-β on Collagen Synthesis by Normal Rat Kidney Epithelial Cells

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The effects of transforming growth factor-β (TGF-β) on the growth of and collagen production by NRK52E cells, a clonal line established from normal rat kidney epithelial cells, have been characterized. NRK52E cells were grown in the absence or presence of TGF-β for 4 days followed by incubation for 24 hours in serum-free medium containing [^{3}H]proline. The secreted and cell-associated collagens produced by control and experimental cultures were isolated by limited pepsin digestion and differential salt fractionation. TGF-β inhibited proliferation by about 50% but did not affect overall culture morphology. Both protein and collagen synthesis were increased in experimental cultures, but the increase in total collagen production exceeded that of total protein synthesis. Although NRK52E cells grown in the presence of TGF-β continued to produce the same types of collagen (types I, III, IV, and V), their relative amounts were changed. In the experimental cultures, type I collagen production was increased eightfold, types III and V collagen levels were increased twofold, but type IV production was only slightly enhanced. In addition to increasing total collagen production by about fivefold, TGF-β increased the ratio of type I to type III about threefold but minimally affected the ratio of secreted to cell-associated molecules. These findings establish that TGF-β specifically affects collagen production in NRK52E cells and that these alterations differ in many ways from the affects of epidermal growth factor. Because TGF-β increased total collagen expression, these results provide additional evidence implicating this growth factor as a positive mediator of matrix accumulation in renal disease. (Am J Pathol 1992, 140:45–55)

The deposition of increased amounts and possibly different types of extracellular matrix (ECM) components characterize the histology of the end-stage kidney.[1–5] Even though ultimately destroying renal function, the molecular basis of this progressive sclerosis remains unresolved. Although the pathogenesis of these changes is likely to be complex,[2,3,6] current evidence implicates certain peptide growth factors and cytokines as mediators of both proliferation and sclerosis in the kidney.[2,3,7–10] Candidate molecules include platelet-derived growth factor, fibroblast growth factor (FGF), epidermal growth factor (EGF), or its functional homolog, transforming growth factor-alpha, transforming growth factor-beta (TGF-β), and the cytokine interleukin-1.[3,8–14] The roles these potential mediators play in both acute and chronic functional and structural changes in the kidney, however, remain incompletely understood.

Several lines of evidence now suggest a role for TGF-β in causing the altered ECM production that occurs during kidney disease. These include: 1) transforming growth factor-β release occurs during platelet activation,[11,15,16] and thus its likely presence at sites of injury in the kidney; 2) transforming growth factor-β affects the growth and state of differentiation of cells and cell lines derived from the kidney[9,17–22]; 3) the control of ECM metabolism and the regulation of expression of the integrin family of receptors that interact with matrix components represent major biologic effects of TGF-β[23–26]; and 4) transforming growth factor-β has been implicated in altered growth and matrix production by renal cells in culture.[9,10,21,27] Additionally recent evidence directly links increased TGF-β levels with development of glomerulonephritis[28,29] and with antiglomerular basement membrane disease[30] in animal models.

Epithelial cells in the kidney likely contribute to the excess and altered matrix accumulation in response to injury.[2,3,10,31] Hence delineation of the effects of growth factors and cytokines, both alone and in combination, on matrix production by renal epithelial cells is relevant for delineating the pathogenetic mechanisms underlying these changes. We have elected to study the effects of certain growth factors on ECM production by NRK52E cells, a permanent clone with a stable collagen pheno-

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type.[32–34] We have recently reported the effects of EGF on cell growth and collagen synthesis by these cells.[34] The present studies evaluate the effects of TGF-β in this system.

Materials and Methods

Materials

The sources for tissue culture plasticware, powdered medium (Dulbecco's modified Eagle's medium [DMEM] containing 4.5 g/liter glucose), tissue culture reagents, fetal bovine serum (FBS), and for biochemical procedures have been previously detailed.[33–36] [^{3}H]Proline (specific activity = 108 Ci/mmol) was purchased from Amersham Corporation, Arlington Heights, Illinois. Transforming growth factor-β1 (porcine) was purchased from R&D Systems, Minneapolis, Minnesota. NRK52E cells were obtained from the American Type Culture Collection; their origin and characterization have previously been detailed.[32–34]

Growth of Cells and Metabolic Labeling

NRK52E cells were grown under standard culture conditions (*control*) or in medium supplemented with 3 ng/ml TGF-β (*Experimental*). In these experiments, all cultures were seeded with the same passage and number (~4 × 10^6 cells/150 mm dish) of cells and were grown simultaneously in medium supplemented with the same lot of FBS. *Control cultures* were grown in 150-mm diameter plastic dishes containing 50 ml standard medium (DMEM supplemented with 10% FBS and 50 μg/ml gentamycin sulfate). The cultures were fed on the third day after subculture by removing the medium and replacing it with an equal volume of fresh standard medium. Four days after subculture, the medium was replaced with 25 ml/dish of labeling medium (DMEM supplemented with 50 μg/ml gentamycin, 1 mg/ml bovine serum albumin, 50 μg/ml ascorbic acid, 100 μg/ml β-aminopropionitrile, and 20 μCi/ml [^{3}H]proline). After incubation for about 24 hours, the clarified culture medium and the acid-soluble fraction derived from the cell layer were prepared as previously described.[33,34,36,37] *Experimental cultures* were grown in the same manner as control cultures, except both the standard and labeling media were supplemented with 3 ng/ml TGF-β. The secreted and cell-associated fractions then were isolated identically to the control cultures. DNA levels were determined by the diphenylamine method[34,36] for control and experimental cultures grown in the same ways described above, except radioactive proline was omitted during the serum-free incubation procedures. All DNA values were determined in triplicate, and the mean values were used in all calculations. In each instance, the standard error of the mean for DNA levels was less than 5%.

Isolation and Characterization of NRK52E Cell Culture Medium and Cell Layer Collagens

The secreted and cell-associated collagens were isolated with the use of pepsin and separated by differential salt fractionation into preparations containing either types I and III or types IV and V collagen molecules using previously detailed procedures.[33,34,36,37] Collagen α-chains were isolated by molecular sieve chromatography on agarose A-5m.[33,38,39] Quantitative analysis was performed by chromatography of the collagen α-chain fractions on carboxymethyl (CM)-Trisacryl (LKB Instruments, Gaithersberg, MD) as previously detailed.[33,34,36,37] All values were normalized to the equivalent amount of DNA in the original cultures. The data presented in this report represent the results obtained in one experimental evaluation of the effects of TGF-β NRK52E cells, which are typical of those obtained in several independent determinations.

Results

Effects of TGF-β on NRK52E Cell Growth

Five days after subculture, control cultures of NRK52E cells displayed a monolayer of cobblestone-shaped cells (Figure 1A). When exposed to TGF-β for 5 days, the overall pattern of cell shape remained the same (Figure 1B). The density of the cells was decreased, however. This decrease in culture density was reflected in an approximate 45% decrease (2.10 mg DNA/10 dishes) in the experimental cultures compared with the DNA content of control cultures, which was 3.85 mg/10 dishes. Thus these studies establish that, as is the case for other epithelial cells,[24,26] TGF-β inhibits NRK52E cell growth.

Effects of TGF-β on Protein and Collagen Synthesis by NRK52E Cells

The effects of exposure to TGF-β on total protein and collagen synthesis were assessed initially by determining the amounts of radioactivity recovered at various points during the isolation of the collagen molecules. Approximations of the rates of total protein synthesis exhibited by NRK52E under each condition were obtained by evaluating the amounts of nondialyzable radioactivity recov-

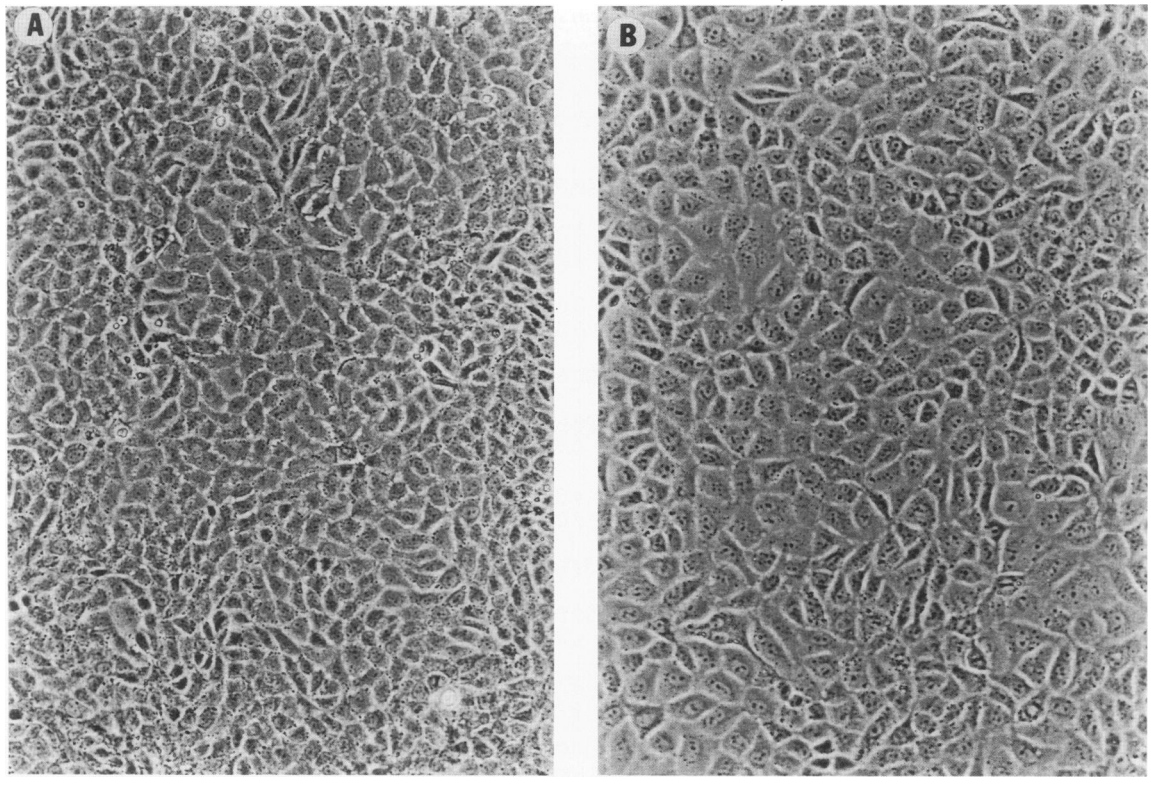

Figure 1. *Phase contrast microscopy of control and NRK52E cell cultures exposed to TGF-β.* **A:** *NRK52E cells grown in the absence of TGF-β.* **B:** *NRK52E cells grown and labeled in the presence of TGF-β. Magnification, ×125.*

ered in the acid extracts of the cell layers and in the $(NH_4)_2SO_4$ precipitates. The amount of radioactivity incorporated into total acid-soluble proteins was increased from 1.6×10^5 cpm/μg DNA (control cultures) to 3.1×10^5 cpm/μg DNA in the experimental cultures. Thus growth in the presence of TGF-β resulted in 90% increase in total cell-associated incorporated radioactivity. Similar levels of increases into cell-associated proteins before pepsin digestion also were observed (Table 1B, line 1). Larger increases in the amounts of radioactivity incorporated into total secreted protein were observed in the experimental cultures (Table 1A, line 1). Overall the amount of radioactivity incorporated into total protein before pepsin digestion was increased 220% in the experimental cultures (Table 1C, line 1). These findings indicate that TGF-β enhanced the rate of total protein synthesis.

To approximate initially the effects of this growth factor on collagen production, the amounts of radioactivity recovered after pepsin-resistant digestion and in the collagen α-chain fractions were evaluated. These evaluations indicated that TGF-β increased the amounts of radioactivity recovered as secreted pepsin-resistant molecules and collagen α-chains fourfold to fivefold (Table 1A, lines 2, 3) and twofold to threefold in the cell-associated fractions (Table 1B, lines 2, 3). Overall TGF-β caused an approximate 2.5- to 3.5-fold increase in the amount of radioactivity incorporated into collagenous proteins (Table 1C, lines 2, 3). Furthermore these analyses indicate a greater stimulation of total collagen production than total protein synthesis. Therefore these initial evaluations indicate that TGF-β specifically enhanced total collagen production.

Quantitative Evaluation of the Effects of TGF-β on Collagen Production by NRK52E Cells

To quantitate the effects of TGF-β on collagen production by NRK52E cells, the collagen chains recovered in each fraction from control and experimental cultures were separately chromatographed on CM-Trisacryl as previously detailed.[33,34,37] Chromatographic analysis of the chains recovered in the types I + III fractions from control (Figure 2A, B) and experimental cultures (Figure 2C, D) showed, in each instance, the presence of type I and type III components. The analysis indicated, however, that TGF-β altered their ratio. The total radioactivity recovered in each chain was determined, and data reduction was performed to establish the amount of radioactivity initially recovered as that genetic type of collagen chain.

Table 1. *Effects of Transforming Growth Factor-β on Protein and Collagen Synthesis by Normal Rat Kidney Epithelial Cells*

	[^{3}H]Proline incorporated (CPM/μg DNA × 10^{-2})	
	Control	+TGF-β (%)
A. Secreted		
1. 0–30% $(NH_4)_2SO_4$	149.5	461.8 (311)
2. Pepsin-resistant		
I + III fraction	4.2	23.1 (550)
IV + V fraction	0.7	2.9 (414)
3. Collagen α chains		
I + III fraction	3.3	17.5 (530)
IV + V fraction	0.3	1.1 (366)
B. Cell-associated		
1. 0–30% $(NH_4)_2SO_4$	790.3	1594.3 (202)
2. Pepsin-resistant		
I + III fraction	28.6	86.5 (302)
IV + V fraction	5.7	12.6 (221)
3. Collagen α chains		
I + III fraction	14.8	44.5 (301)
IV + V fraction	2.0	4.4 (220)
C. Total		
1. 0–30% $(NH_4)_2SO_4$	939.8	2056.1 (219)
2. Pepsin-resistant		
I + III fraction	32.8	109.6 (334)
IV + V fraction	6.4	15.5 (242)
3. Collagen α chains		
I + III fraction	18.1	62.0 (343)
IV + V fraction	2.3	5.5 (239)

Incorporation of [^{3}H]proline in the absence or presence of 3 ng/ml TGF-β into each fraction was determined as described in Materials and Methods. Line 1: radioactivity recovered in the 0–30% $(NH_4)_2SO_4$ fractions. Line 2: radioactivity recovered in the 4.5 M NaCl insoluble fraction after dialysis against 0.5 M HOAc/0.9 M NaCl. Line 3: radioactivity recovered as collagen α chains after chromatography of the types I + III and types IV + V fractions on agarose A-5m. The values in parentheses represent the percent of the radioactivity recovered in a fraction from NRK52E cells exposed to TGF-β relative to the corresponding control value.

These evaluations (Table 2A, B) indicated that the amount of radioactivity recovered in type III molecules was increased ~2.5-fold in both the secreted and cell-associated fractions derived from the experimental cultures. In contrast, the production of type I molecules was enhanced more than 10-fold in the secreted molecules and ~7.5-fold in the cell-associated molecules. Thus although TGF-β increased production of both collagen types, it differentially affected their expression.

We have previously documented that NRK52E cells produce type IV molecules containing the α1(IV) chain and type V molecules containing α1(V), α2(V), and α3 (V) components.[33] CM-Trisacryl chromatographic evaluations of the chains recovered in the secreted and cell-associated types IV + V fractions from control (Figure 3A, B) and experimental cultures (Figure 3C, D) established that the cells continued to produce molecules containing the same profile of type IV and type V components when grown in the presence of TGF-β. After normalization to DNA levels followed by data reduction (Table 2A, B), these analyses indicated that TGF-β minimally altered the production of molecules containing the α1(IV) chain but increased production of molecules containing type V components. Thus TGF-β again differentially affected the expression of these two collagen types.

Summation of the amounts of radioactivity corresponding to each genetic type of collagen chain recovered in the different fractions (Table 2C) indicated that exposure of NRK52E cells to TGF-β resulted in collagen type-specific changes. These results are summarized in Figure 4. The relative amount of total type I collagen produced was increased eightfold, type III synthesis was increased ~2.5-fold, type IV production was essentially unchanged, and the amount of radioactivity recovered in type V molecules was increased ~80%. Overall total collagen production was enhanced almost fivefold on prolonged exposure to this growth factor (Table 2C and Figure 4).

We have previously documented that EGF alters the compartmental distribution (increases the ratio of secreted to cell-associated molecules) of the collagen types produced by NRK52E cells.[34] To evaluate this possibility for TGF-β, the ratios of the amounts of radioactivity recovered in the secreted and cell-associated fractions for each type of collagen were calculated from the data in Table 2, and these results are presented in Table 3. Exposure to TGF-β resulted in an increase in the ratio of secreted to cell-associated type I molecules but a decrease in this ratio for types III, IV, and V molecules. Because type I collagen constituted the major collagen type produced by NRK52E cells exposed to TGF-β, a slight increase (~10%) in the ratio of secreted to cell-associated total collagen occured.

Our previous studies have demonstrated an ~50%

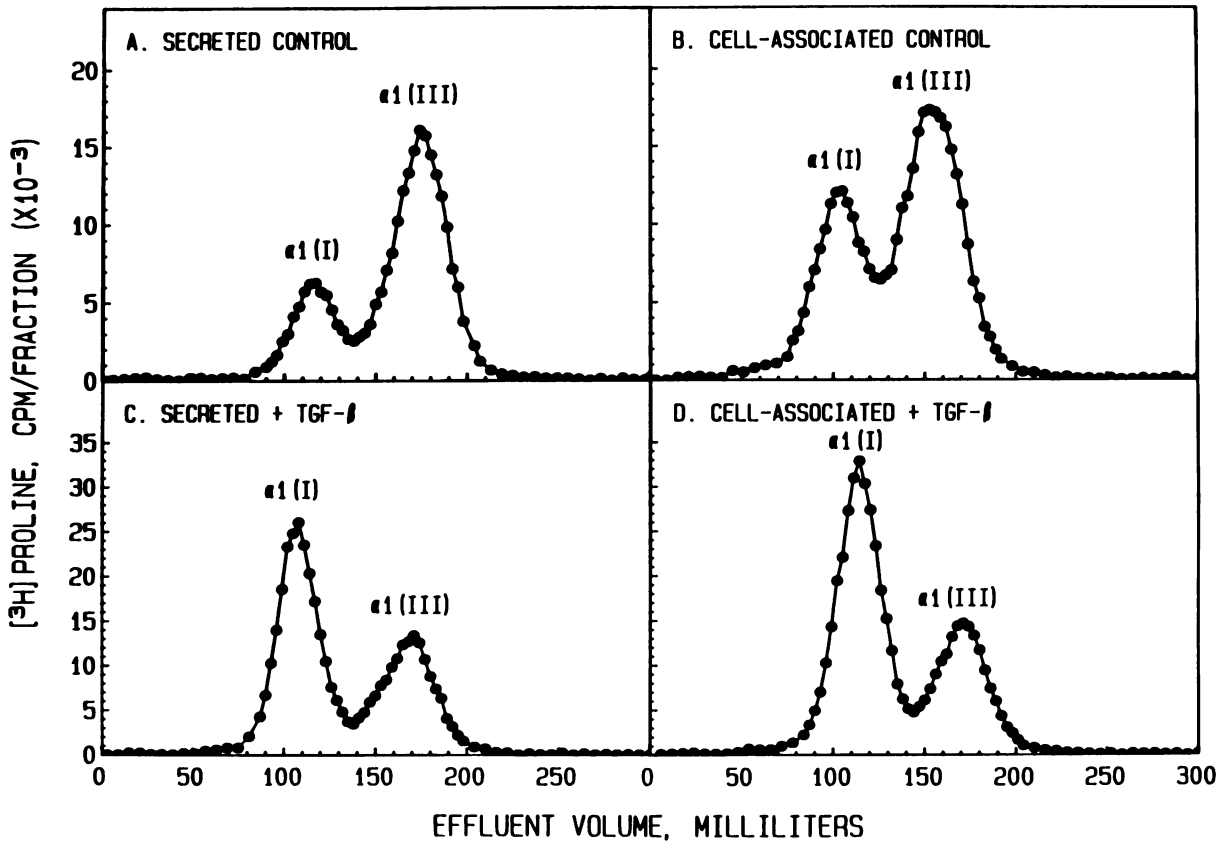

Figure 2. *Carboxymethyl-Trisacryl chromatographies of collagen chains recovered in the types I + III fractions synthesized by control and NRK52E cells grown in the absence or presence of TGF-β.* A: *An aliquot (349,000 cpm) of collagen α chains recovered after agarose chromatography of the secreted types I + III fractions from control NRK52E cell cultures was chromatographed.* B: *An aliquot (529,000 cpm) of collagen α chains recovered after agarose chromatography of the cell-associated types I + III fractions from control NRK52E cell cultures was chromatographed.* C: *An aliquot (537,000 cpm) of collagen α chains recovered after agarose chromatography of the secreted types I + III fractions from NRK52E cells chronically exposed to TGF-β was chromatographed.* D: *An aliquot (622,000 cpm) of collagen α chains recovered after agarose chromatography of the cell-associated types I + III fractions from NRK52E cells chronically exposed to TGF-β was chromatographed. The amounts of radioactivity eluting in the positions corresponding to either the α1(I) or α1(III) chains, respectively, were individually determined for each chromatography to establish the fraction of total radioactivity recovered as the genetic type of collagen chain. These values are presented in Table 2.*

increase in the ratio of type I-homotrimer to type III collagen results from exposure of NRK52E cells to EGF.[34] The data presented in this report indicate that TGF-β differentially affects the amounts of radioactivity recovered in these genetic types of collagen (Table 2 and Figures 2, 4). To quantify these changes, the ratios of type I-homotrimer to type III collagen were calculated, and these results are presented in Table 4. Exposure to TGF-β increased the ratio of type I-homotrimers to type III molecules in both the secreted and cell-associated fractions. Overall, these changes resulted in an ~300% increase in this ratio.

Discussion

The results presented herein indicate that TGF-β inhibits growth but increases total protein synthesis in a cell culture model of normal renal epithelial cells. Additionally several changes in collagen metabolism occurred in response to this growth factor. These include: 1) an enhancement of total collagen production; 2) a differential enhancement of expression of the different collagen types with the production of type I collagen increased to the greatest extent and the synthesis of type IV molecules being the least affected; and 3) a substantial increase in the ratio of type I to type III collagen. Furthermore the increase observed in total collagen production exceeded the increase in total protein synthesis. Thus these findings establish that TGF-β specifically and differentially affects collagen production in NRK52E cells. It should be noted that our conclusions regarding the effects of this growth factor on collagen synthesis are based on values normalized to the amount of DNA in the original cultures. Hence our values reflect the amount of radioactivity recovered in a specific genetic type of collagen or in total collagen per cell. Even though there are ~50% fewer cells in the experimental cultures, total collagen production per cell is increased more than fivefold (Table 2). Thus total collagen accumulation is enhanced by exposure to TGF-β.

Table 2. *Summary of Carboxymethyl–Trisacryl Chromatographies of Collagen Chains Synthesized by NRK52E Cells in the Absence or Presence of TGF-β*

	[^{3}H]Proline incorporated (CPM/μg DNA)	
	Control	+TGF-β (%)
A. Secreted molecules		
α1 (I)	84	886 (1055)
α1 (III)	132	322 (244)
α1 (IV)	1	1 (100)
α1 (V)	9	7 (78)
α2 (V)	7	6 (86)
α3 (V)	4	3 (75)
B. Cell-associated molecules		
α1 (I)	408	3048 (747)
α1 (III)	403	1051 (258)
α1 (IV)	8	10 (125)
α1 (V)	46	99 (215)
α2 (V)	41	86 (210)
α3 (V)	38	59 (155)
C. Total collagen synthesized		
α1 (I)	492	3934 (800)
α1 (III)	535	1380 (258)
α1 (IV)	9	11 (122)
α1 (V)	53	105 (198)
α2 (V)	50	93 (186)
α3 (V)	42	62 (148)
Total	1181	5585 (473)

Values presented were calculated from the amounts of radioactivity recovered from the appropriate areas of the corresponding CM-Trisacryl chromatographies (Figures 4 & 5) of the collagen α chains recovered in the indicated fraction. The numbers in parentheses are the percentage of the radioactivity recovered in a fraction from the cells grown in medium supplemented with TGF-β relative to that in the corresponding control fraction.

Therefore these data provide further evidence implicating TGF-β as a positive mediator involved in the excess collagen production and accumulation that occurs in many renal diseases.

It is now well accepted that one of the major effects of TGF-β is the regulation of ECM production.[24,26,40] Transforming growth factor-β increases type I collagen production in normal rat kidney fibroblasts,[17] stimulates total collagen production in chick embryo fibroblasts,[41] in human dermal fibroblasts,[42] in rat osteoblastlike cells,[43] and in fetal calvarial bone cells.[44] In contrast to our findings in this system, in which TGF-β alters the relative proportion of type I to type III molecules, this growth factor has been reported to increase the production of both types I and III collagen without altering their relative amounts in human fetal lung fibroblasts.[45] While stimulating collagen biosynthesis in articular chondrocytes,[46] TGF-β decreases collagen synthesis in growth plate chondrocytes.[40] Our conclusion that the changes in collagen production by NRK52E cells in response to TGF-β reflect a selective effect on collagen synthesis rather than being a consequence of changes in total protein production is based on the following observations. First, the degree of stimulation differed for each genetic type of collagen examined (Table 2 and Figure 4). Second, total protein synthesis was stimulated ~2-fold (Table 1), but total collagen production was increased almost fivefold (Table 2). In human gingival fibroblasts, the increase in collagen production resulting from TGF-β exposure has been reported to parallel that of total protein production.[47] In human fetal lung[45] and normal rat kidney fibroblasts,[48] in human intestinal smooth muscle cells,[49] and in fetal rat lung epithelial cells,[50] however, similar selective increases in total collagen production resulting from TGF-β exposure have been documented. Thus our findings further support the concept that collagen production is specially enhanced by TGF-β and that this phenomenon occurs in renal epithelial cells.

Transforming growth factor-β inhibits glomerular endothelial, epithelial, and mesangial cell proliferation.[21] Additionally this growth factor has been reported to stimulate collagen[21] and proteoglycan[27] synthesis in mesangial cells and to promote fibronectin accumulation in glomerular epithelial cells.[21] Furthermore, as a general rule, TGF-β inhibits proliferation of epithelial cells,[26] and this growth inhibition correlates with increased matrix, particularly collagen, accumulation. Recent evidence has directly linked enhanced collagen production resulting from TGF-β treatment with growth inhibition in kidney fibroblasts.[17] Although our data are consistent with this concept, several instances of an increase in collagen production without associated growth inhibitory effects also have been reported.[45,49,50] Additionally evidence suggests that the stimulation of ECM and proto-oncogene gene expression by TGF-β are controlled through different pathways.[51] Thus the relationship be-

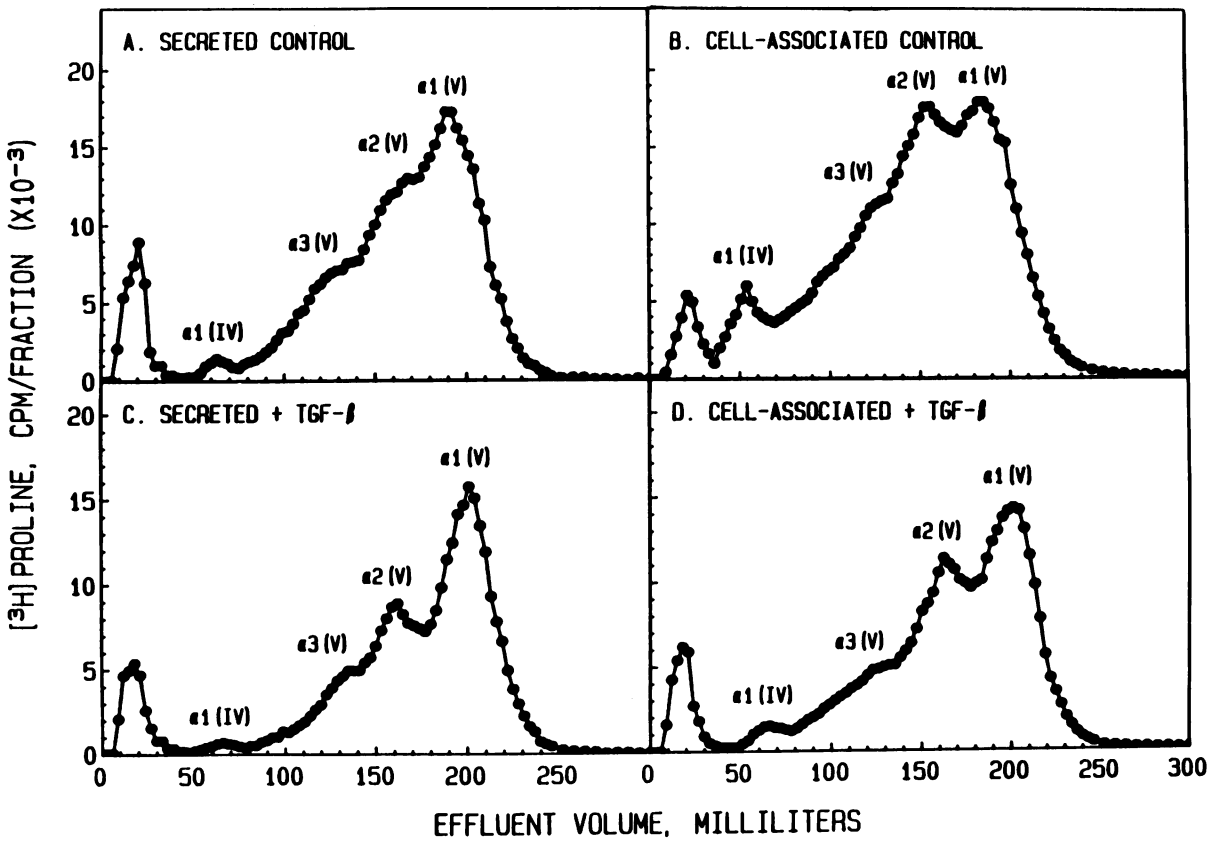

Figure 3. *Carboxymethyl-Trisacryl chromatographies of collagen chains recovered in the types IV + V fractions synthesized by NRK52E cells grown in the absence or presence of TGF-β.* A: *An aliquot (684,000 cpm) of collagen α chains recovered after agarose chromatography of the secreted types IV + V fractions from control NRK52E cell cultures was chromatographed.* B: *An aliquot (735,000 cpm) of collagen α chains recovered after agarose chromatography of the cell-associated types IV + V fractions from control NRK52E cell cultures was chromatographed.* C: *An aliquot (398,000 cpm) of collagen α chains recovered after agarose chromatography of the secreted types IV + V fractions from NRK52E cells chronically exposed to TGF-β was chromatographed.* D: *An aliquot (465,000 cpm) of collagen α chains recovered after agarose chromatography of the cell-associated types IV + V fractions from NRK52E cells chronically exposed to TGF-β was chromatographed. The amounts of radioactivity eluting in the positions corresponding to either the α1(IV), α3(V), α2(V) or α1(V) chains, respectively, were individually determined for each chromatography to establish the fraction of total radioactivity recovered as the genetic type of collagen chain. These values are presented in Table 2.*

tween increased matrix production and inhibition of proliferation remains controversial.

In addition to indicating that TGF-β may be a positive effector of collagen production in this renal epithelioid cell line, our studies indicated that chronic exposure to TGF-β did not significantly alter the culture distribution (secreted *versus* cell-associated) of the collagens synthesized by NRK52E cells (Table 3). In fact, with the exception of type I collagen, this growth factor slightly promoted the retention of the other types of collagen by the cells. Furthermore, for the most part, the changes observed in the relative amounts of the different collagen types secreted into the medium paralleled those in the cell layers (Table 2 and Figure 4). Because the magnitude of stimulation differed for each collagen type, these findings suggest that TGF-β alters the collagen composition of the matrix surrounding the cells. Alterations in the composition of the ECM resulting from the action of TGF-β also have been described in chick fibroblasts.[52] Because the composition of the extracellular matrix not only governs normal cellular function but also its response to injury,[2,3] it can be speculated that this altered collagen profile may create the milieu for the progression of tubular fibrosis.

Our findings show also that TGF-β stimulates the expression of type I molecules to a greater extent than it does type III collagen. Consequently the ratio of type I to type III molecules produced by NRK52E cells is increased (Table 4). Changes in the ratio of type I to type III collagen have been associated with wound healing. The selective stimulation of type I collagen by TGF-β increases this ratio in NRK52E cells more than threefold. As an increased proportion of type I collagen is generally associated with fibrotic responses,[35] this finding also suggests TGF-β promotes the phenotype of an epithelial cell undergoing fibrosis.

Although this report focuses on the isolated effects of TGF-β, clearly this peptide does not impact on cells *in vivo* in the absence of other factors, such as platelet-

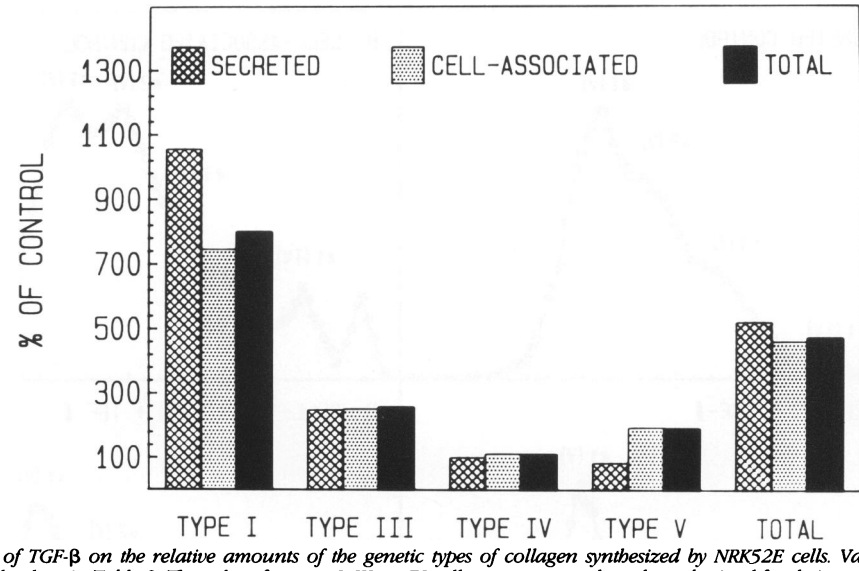

Figure 4. *Effects of TGF-β on the relative amounts of the genetic types of collagen synthesized by NRK52E cells. Values presented were calculated from the data in Table 2. The values for types I, III, or IV collagen represent the values obtained for their respective chains. Those for type V collagen are the sums of the radioactivities obtained for the α1(V), α2(V), and α3(V) chains. The values calculated for each collagen type produced by the cells grown in the presence of TGF-β were normalized to the value calculated for the corresponding type of collagen synthesized by the control cultures.*

derived growth factor (PDGF), interleukin-1, EGF, and FGF.[3,11–14] In this regard, it should be noted that the effects of TGF-β differ markedly from those previously reported for EGF on the NRK52E cells.[34] Comparison of the effects of these peptides (Table 5) indicates that they affect both growth and total collagen production in directly opposite ways. First, whereas the production of type III is the most sensitive to EGF, type I collagen production is most affected by TGF-β. Second, EGF results in a more than 10-fold increase in the ratio of secreted to cell-associated molecules, but TGF-β minimally impacts this aspect of collagen metabolism. Finally, whereas the ratio of type I to type III collagen is increased by about 50% by EGF, this ratio is enhanced about threefold by TGF-β. Similar disparate effects of these growth factors on collagen production have previously been documented in both kidney fibroblasts[48] and fetal rat lung epithelial cells.[50] The actions of TGF-β on renal epithelial cells likely do not occur in a milieu devoid of other growth factors and cytokines. Because we have now documented the disparate effects of EGF and TGF-β in this system, further investigations into the effects of TGF-β in

Table 3. *Effects of TGF-β on the Culture Distribution of the Genetic Types of Collagen Synthesized by NRK52E Cells*

	[^{3}H]Proline incorporated (CPM/μg DNA)		
	Secreted	Cell-associated	Ratio
Type I collagen			
Control	84	408	0.21
+TGF-β	886	3048	0.29
Type III collagen			
Control	132	403	0.33
+TGF-β	322	1051	0.31
Type IV collagen			
Control	1	8	0.12
+TGF-β	1	10	0.10
Type V collagen			
Control	20	125	0.16
+TGF-β	16	244	0.07
Total collagen			
Control	240	944	0.25
+TGF-β	1225	4353	0.28

Values presented were derived from the data in Table 2. Values for the amounts of type V collagen are the sum of the amounts of radioactivities recovered in the α1(V), α2(V), and α3(V) fractions. Ratio is the ratio of secreted to cell-associated radioactivity recovered in the indicated preparation.

Table 4. *Effects of TGF-β on the Amounts of Type I and Type III Collagen Synthesized by NRK52E Cells*

	[^{3}H]Proline incorporated (CPM/μg DNA)		
	Type I	Type III	Ratio
Secreted molecules			
Control	84	132	0.6
+TGF-β	886	322	2.75 (430%)
Cell-associated molecules			
Control	408	403	1.01
+TGF-β	3048	1051	2.90 (287%)
Total			
Control	492	535	0.92
+TGF-β	3934	1373	2.86 (311%)

Values presented were derived from the data in Table 2. Ratio is the ratio of the amounts of radioactivity recovered in type I-homotrimer molecules to that recovered in type III molecules in the indicated preparation. Numbers in parenthesis represent the percent change from control values in the ratios.

combination with other factors on matrix production are required for understanding the overall role of TGF-β in renal disease.

Increased synthesis and deposition of ECM components characterize the end-stage of many renal diseases.[2–5] In addition to the recent evidence indicating that TGF-β affects growth and matrix production by glomerular cells,[9,21,22] altered levels of this growth factor have been directly linked to the pathogenesis of an animal model of glomerulonephritis.[28,29] Although most studies have focused on the glomerulus, it should be noted that fibrosis and tubular sclerosis are likewise structural hallmarks of many renal pathologies, and interstitial fibrotic disease is an almost constant companion of chronic renal diseases.[53] Our studies have employed NRK52E cells as a model for investigating the effects of growth factors for several reasons. First, others have indicated that NRK52E cells are derived from the normal tubular epithelial cell.[32] Second, we have previously demonstrated their stable collagen phenotype[33] that differs from that reported for the glomerular epithelial cell, which essentially only expresses type IV collagen in culture.[54–56] The stable collagen phenotype offers the advantage of minimizing possible variations in collagen production, which have been reported for primary cultures of renal epithelial cells,[57] that could obscure growth factor effects. It should be noted that the collagen phenotype (expression of types I, III, IV, and V collagens[33]) of NRK52E cells is similar to that of MCT cells, which were derived from proximal tubular epithelial cells.[58] Likewise the normal morphology of NRK52E cells (Figure 1A) resembles that of primary cultures of rabbit proximal tubule cells,[10] and TGF-β inhibits also the proliferation of primary cultures of rabbit proximal tubule cells.[10] However TGF-β induces a clustering of rabbit proximal tubule epithelial cells in culture,[10] a change that did not occur with NRK52E cells (Figure 1B). Thus, although the properties of NRK52E cells suggest a renal tubule epithelial cell origin, clearly the cells have adapted in culture, and hence the relevance of our findings to the issue of tubular fibrosis remains speculative. Regardless the findings presented herein and our previous results[34] establish the initial paradigm for comparing the effects of TGF-β with those of other growth factors on collagen production by an epithelial cell of renal origin.

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Table 5. *Comparison of the Effects of TGF-β and EGF on NRK52E Cells*

	Growth factor	
	TGF-β*	EGF†
Growth	Inhibited	Stimulated
Total collagen production	Increased	Decreased
Collagen type most affected	Type I	Type III
Ratio of secreted to cell-associated molecules	Increased ~10%	Increased ~10-fold‡
Ratio of type I to type III collagen molecules	Increased ~300%	Increased ~50%‡

* Data presented in this report.
† Data from [34].
‡ Result obtained under conditions of chronic exposure to EGF.

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