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Keratinocyte growth factor functions in epithelial induction during seminal vesicle development

(androgen/epithelial–mesenchymal interactions/branching morphogenesis)

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ABSTRACT **Development of the seminal vesicle (SV) is elicited by androgens and is dependent on epithelial–mesenchymal interactions. Androgenic signal transmission from the androgen-receptor-positive mesenchyme to the epithelium has been postulated to involve paracrine factors. Keratinocyte growth factor (KGF), a member of the fibroblast growth factor family, is produced by stromal/mesenchymal cells and acts specifically on epithelial cells. The KGF transcript was detected by reverse transcription–polymerase chain reaction in newborn mouse SVs and by Northern blot analysis of RNA from cultured neonatal SV mesenchymal cells. Newborn SVs placed in organ culture undergo androgen-dependent growth and differentiation. Addition of a KGF-neutralizing monoclonal antibody to this system caused striking inhibition of both SV growth and branching morphogenesis. This inhibition was due to a decline in epithelial proliferation and differentiation, as the mesenchymal layer was not affected by anti-KGF treatment. When KGF (100 ng/ml) was substituted for testosterone in the culture medium, SV growth was ≈50% that observed with an optimal dose of testosterone (10^{-7} M). All of these findings suggest that KGF is present during a time of active SV morphogenesis and functions as an important mediator of androgen-dependent development.**

Seminal vesicles (SVs) are male sex-accessory glands derived from the caudal regions of the Wolffian ducts. The development and maintenance of the SV are dependent on epithelial–mesenchymal interactions that are directed by androgens (1–4). SV morphogenesis begins on embryonic day 15 in mice (5) subsequent to the onset of testosterone production by the fetal testes (6). Fetal development of the SV is driven by androgens acting directly on the mesenchyme that induces epithelial development. The mesenchyme must initially mediate the inductive action of androgens because it is the only source of androgen receptor until day 1 postnatally, when epithelial androgen receptors are first detected (7). This conclusion is reinforced by analysis of SV inductions employing normal androgen receptor-positive SV mesenchyme and androgen receptor-negative epithelium (testicular feminization) (8). Dependence on epithelial–mesenchymal interaction continues into adulthood, as demonstrated by homotypic and heterotypic recombination experiments. SV mesenchyme is capable of inducing SV differentiation, morphologically and functionally, from epithelia isolated from any portion of the fetal Wolffian duct (3, 4), adult epididymis (9), and adult ureter and adult ductus deferens (2). Thus, both prenatal and postnatal development depends on instructive signals from the mesenchyme to the epithelium.

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The identification of paracrine signals that mediate these cell–cell interactions has proven elusive. Recently, a growth factor whose properties are consistent with such an effector has been identified and molecularly cloned. This factor, designated keratinocyte growth factor (KGF), is a heparin-binding growth factor and member of the fibroblast growth factor (FGF) family (FGF-7) (10). KGF is secreted by mesenchymal cells and appears to act solely on epithelial cells (11, 12). The present studies were undertaken to investigate the possibility that KGF is an important mediator of the epithelial–mesenchymal interactions required for androgen-dependent SV development.

MATERIALS AND METHODS

Animals and Organ Culture. SVs were dissected from newborn BALB/c mice obtained from either the Cancer Research Laboratory (University of California, Berkeley) or Simonsen Laboratories (Gilroy, CA). SVs were grown in an organ culture system as described (13). Briefly, the organs were placed on Millipore filters supported on triangular metal grids in 35-mm Petri dishes (Falcon). A basal medium of Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12, 1:1 (vol/vol), supplemented with insulin (10 μg/ml), transferrin (10 μg/ml), cholera toxin (10 ng/ml), bovine serum albumin (5 mg/ml; Sigma), gentamycin (50 μg/ml), and epidermal growth factor (EGF; 10 ng/ml; Collaborative Research) was utilized in all experimental groups. (Preliminary studies indicate that cholera toxin, bovine serum albumin, and EGF are nonessential components of the medium, as a simplified medium supplemented only with insulin, transferrin, and testosterone is sufficient to promote optimal growth and development of the neonatal mouse SV.) Where indicated, either KGF monoclonal antibody (mAb) or a control mouse IgG mAb (Sigma) was added to 6 μg/ml. Testosterone and KGF were included in some experimental groups as indicated. The medium was changed every 2 days.

KGF and KGF mAb. Human KGF was generated with a T7 vector bacterial expression system and purified by heparin-Sepharose chromatography to ≈90% homogeneity as judged by silver staining and immunoblot analysis of SDS/polyacrylamide gels, as well as mitogenic-specific activity relative to KGF standards quantified by amino acid analysis (14). The KGF murine mAb designated 2C4 was prepared by using the recombinant human KGF as antigen. mAb 2C4 IgG was purified from mouse ascites fluid with GammaBind resin

Abbreviations: SV, seminal vesicle; KGF, keratinocyte growth factor; mAb, monoclonal antibody; FGF, fibroblast growth factor; EGF, epidermal growth factor.

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(Pharmacia) by following manufacturer's instructions and stored either frozen or refrigerated at 6 mg/ml. The specificity of 2C4 neutralizing activity was demonstrated in an assay of [^{3}H]thymidine incorporation by BALB/MK cells (12) treated with KGF, acidic FGF (FGF-1, recombinant human, from Upstate Biotechnology), basic FGF (FGF-2, recombinant human, from Upstate Biotechnology), or EGF (recombinant human, from Collaborative Research).

Measurement of DNA Content. SVs were harvested and stored frozen at −70°C in 1.5-ml tubes until DNA assays were performed. The samples were thawed in 0.5 M NaOH, sonicated, and incubated at 60–65°C for 4 h. After incubation, the solution was neutralized with 0.5 M HCl, and DNA content was measured using Hoechst dye (15). Statistical differences were determined using Student's *t* test for independent samples.

Image Analysis. Images of whole-mount photographs of SVs were captured and digitized with a Dage-MTI CCD-72 TV camera interfaced with a Macintosh IIcx computer and processed with PRISM VIEW software (Dapple, Sunnyvale, CA). PRISM VIEW software calculated epithelial and total area from computer-generated binary images of individual samples. To determine mesenchymal area, the epithelial area was subtracted from the total area. Morphological complexity of the epithelial shape was assessed using the node number parameter of PRISM VIEW, which is a topological measure whose value is proportional to the complexity of ductal branching (16). The node number is determined from a computer-generated skeletonized epithelial outline and defined as the number of internal connections between three or more links in the skeleton. Statistical differences for growth and morphological data were determined using Student's *t* test for independent samples.

Reverse Transcription–PCR. RNA was isolated from the SVs of newborn mice utilizing the method of Chomczynski and Sacchi (17). After phenol/chloroform extraction, RNA in the aqueous phase was precipitated with ethanol and dried. The pellet was reconstituted in 80 μl of reverse transcriptase buffer consisting of 50 mM Tris·HCl (pH 8.3), 6 mM $MgCl_2$, 40 mM KCl, 20,000 units of RNase inhibitor (Boehringer Mannheim), all four dNTPs (each at 1 mM), 100 pmol of random hexamers, 20 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim), and diethylpyrocarbonate (DEPC)-treated H_2O. The solution was incubated at 23°C for 10 min, 42°C for 90 min, 95°C for 5 min, and cooled immediately to 4°C. The product was ethanol-precipitated and the resultant pellet was reconstituted in 500 μl of DEPC-treated H_2O. The PCR was carried out with 50 μl of reverse transcription product in PCR buffer containing 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, all four dNTPs (each at 0.20 mM), each of the 5′ and 3′ primers at 1 μM, and DEPC-treated H_2O to give a total volume of 100 μl (18). The 5′ primer was 5′-ATACTGACATGGATCCTGCCA-3′ and the 3′ primer was 5′-ATAAGGTGTTATAATGGTTTTC-3′. These primers flank the coding region of mouse KGF from nt 22 to 465 (D.R., unpublished observations). The amplification reaction was performed in a programmable cyclic reactor (Ericomp, San Diego) with an initial cycle of 60°C for 15 min prior to the addition of 1 unit of *Taq* polymerase (AmpliTaq, Cetus), followed by 60 cycles, each consisting of the sequential temperatures and time durations as follows: 94°C for 30 sec, 45°C for 30 sec, and 72°C for 2 min. A final cycle at 72°C for 45 min was performed to extend any unfinished amplified strands.

Southern Blot Analysis. The PCR-amplified DNA was electrophoresed in 2% SeaKem agarose (FMC) and visualized using ethidium bromide staining. The DNA was transferred to Genetrans nylon membrane by the method of Maniatis *et al.* (19). Filters were prehybridized for 2 h at 42°C in Hybrisol [Oncor; 50% (vol/vol) formamide/10% (wt/vol) dextran sulfate/1% SDS/6× standard saline citrate (SSC)/herring DNA] and hybridized at 42°C for 20 h in the same solution with a [^{32}P]dCTP-labeled probe corresponding to the coding sequence of mouse KGF. Filters were washed for two 30-min periods at room temperature in 2× SSC/1% SDS and for two 30-min periods at 50°C in 0.1× SSC/1% SDS and exposed to Kodak X-Omat AR film.

Northern Blot Analysis. The epithelial and mesenchymal layers of isolated SVs were manually separated after a 90-min treatment with 1% trypsin at 4°C (2). The SV mesenchyme was collected from 12 neonatal mice (24 SVs) and cultured for 7 days in serum-containing medium consisting of a 1:1 mixture of DMEM and Ham's F-12 with 10% (vol/vol) fetal bovine serum and gentamycin (50 μg/ml). Previous studies (2) demonstrated that >95% of isolated mesenchymes show no evidence of epithelial contamination. During the culture period, inspection of the culture dishes revealed cells of mesenchymal/fibroblastic morphology without any evidence of colonies with epithelial morphology. The cell culture components and medium were obtained from the University of California Cell Culture facility (San Francisco). Upon reaching ≈90% confluency, the cells were thoroughly rinsed with phosphate-buffered saline (PBS) and the medium was replaced by serum-free medium containing DMEM/Ham's F-12 supplemented with insulin (5 μg/ml) and bovine serum albumin (5 mg/ml) in the presence or absence of testosterone (10^{-7} M; Sigma). The culture fluid was discarded after 1 day and replaced with fresh medium. After an additional 3 days, the cells were harvested and frozen for RNA analysis. NIH 3T3 murine embryonic lung fibroblasts (20) and BALB/MK murine keratinocytes (21) were used as positive and negative controls, respectively. Cells were grown in 60-mm tissue culture dishes, washed with PBS, pelleted by low-speed centrifugation, and stored at −70°C. Frozen pellets were homogenized in the presence of RNAZol (Tel-Test, Friendswood, TX) and extracted with chloroform. RNA was recovered by precipitation with ice-cold isopropanol. RNA (10 μg) was electrophoresed on 1% formaldehyde/agarose gels and transferred to Nytran nylon membranes. Filters were hybridized and washed as described above. Densitometric analysis was performed using a Bio-Rad scanner and a Pharmacia Ultroscan XL laser densitometer with GELSCAN XL software. After screening for KGF expression, filters were hybridized with a mouse β-actin cDNA probe to standardize for RNA loading and transfer.

RESULTS

Detection of KGF in SV. To implicate KGF involvement in mouse SV development, it was necessary to first establish its presence in the organ during a period of active morphogenesis. For this purpose, RNA from intact SVs of newborn animals was reverse-transcribed, PCR-amplified, and screened by Southern blot analysis with a mouse KGF cDNA probe. Fig. 1*A* shows a single band, consistent in size with a fragment generated from the KGF sequence (443 bp) and matching the band seen with amplified RNA from KGF-expressing NIH 3T3 cells. These results demonstrated that the KGF transcript was produced in neonatal SVs. Northern blot analysis of RNA from primary cultures further established that SV mesenchymal cells expressed KGF RNA and that the KGF transcript level increased 2.5-fold in response to testosterone (Fig. 1*B*).

KGF-Neutralizing mAb Inhibits Branching Morphogenesis in the SV. Growth and development of the mouse SV in organ culture mimic the process observed *in situ*, including an absolute dependence on androgen (13). As shown in Fig. 2*B*, the SV morphology maintained in the absence of testosterone was very similar to that of the SV freshly dissected from the

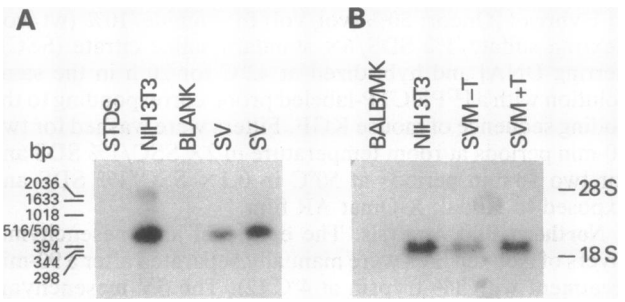

FIG. 1. KGF expression in neonatal mouse SVs. (*A*) RNA from NIH 3T3 cells and two SV whole-organ samples (each consisting of six organs) was reverse-transcribed and PCR-amplified. The products were gel electrophoresed, transferred to nylon membrane, and hybridized to a ^{32}P-labeled probe corresponding to the mouse KGF coding region. No specimen was loaded in the lane labeled BLANK. The lane designated STDS contained DNA size markers whose positions are shown at left. (*B*) RNA was isolated from BALB/MK keratinocytes, NIH 3T3 fibroblasts, and SV mesenchymal cell cultures (SVM) incubated for 96 h in the presence (+) or absence (−) of testosterone (T). Total RNA (10 μg) was subjected to Northern blot analysis with a ^{32}P-labeled mouse KGF probe. To control for loading and transfer, the same blots were hybridized with a mouse β-actin cDNA probe (data not shown). The location of 28S and 18S rRNA is indicated.

newborn animal (Fig. 2*A*). The epithelial structure had a simple cane shape with no projections or infoldings. After 4 days of culture in the presence of testosterone, the epithelium had become elongated and its border was folded, typical of SV morphogenesis (Fig. 2*C*). By 6 days, the branching became so complex that the highly convoluted epithelial border of the epithelium was difficult to discern (Fig. 2*E*).

To dissect the role of KGF in SV morphogenesis using the organ culture model, we employed a KGF-specific neutralizing mAb. As shown in Table 1, mAb 2C4 blocked the activity of KGF in a [^{3}H]thymidine incorporation bioassay but did not inhibit other mitogens, including EGF and acidic and basic FGFs. It also failed to neutralize FGF-8 (22), a recently identified androgen-inducible growth factor expressed by the rat Shionogi mammary carcinoma line (data not shown). SV incubation in the presence of 2C4 resulted in a dramatic reduction in branching morphogenesis observed both at 4 and 6 days (Fig. 2 *D* and *F*). Under the same conditions, normal development was observed with a control mouse mAb (Fig. 2 *C* and *E*). To quantify the morphological complexity of epithelium obtained in these conditions, analysis of node number was performed on individual SV specimens by image analysis. The node number is indicative of the number of branches of a skeletonized image derived from a euclidian distance map. Thus, the greater the number of epithelial folds or branches (i.e., morphological complexity), the greater the number of nodes. This parameter was determined automatically by PRISM VIEW software on SVs grown for 4 and 6 days. As shown in Table 2, epithelium of SVs grown without testosterone demonstrated only 0.2 ± 0.2 node. The mean number of nodes at 4 days calculated for SV in the presence of control IgG plus testosterone was 10.7 ± 2.4, and exposure to anti-KGF plus testosterone resulted in a marked reduction to 2.4 ± 0.1 nodes. The striking differences in node number persisted at 6 days as well (Table 2). Preliminary experiments using 1G4, a different KGF mAb (23), yielded results comparable to those obtained with 2C4 (data not shown). These findings established that epithelium of SVs incubated in the presence of anti-KGF showed significantly less androgen-dependent morphological complexity than tissue exposed to the control antibody.

Anti-KGF Inhibits Growth of SV in Organ Culture. After birth, the SV *in situ* and in organ culture undergoes an

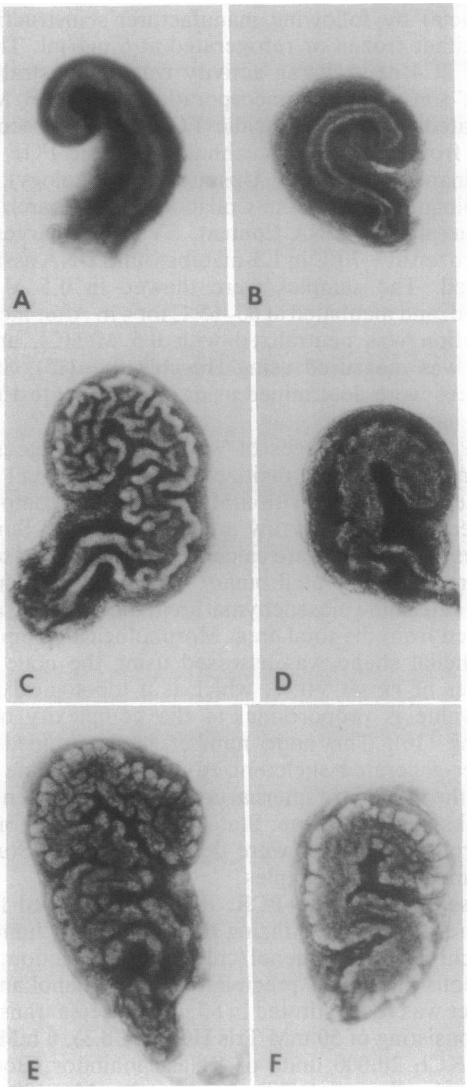

FIG. 2. Effect of anti-KGF on SV morphology. Representative whole-mount photographs of SVs grown in organ culture with medium supplemented as follows: (*A*) Basal medium (day 0). (*B*) Mouse control IgG (6 μg/ml) for 6 days. (*C*) Mouse control IgG and testosterone (10^{-7} M) for 4 days. (*D*) Anti-KGF (6 μg/ml) plus testosterone (10^{-7} M) for 4 days. (*E*) Mouse control IgG plus testosterone for 6 days. (*F*) Anti-KGF plus testosterone for 6 days. The epithelial layer, which lines the lumen of the gland, is seen as the inner light layer. As morphogenesis progresses, this inner layer becomes increasing convoluted. The mesenchymal layer is the outer dark layer, which defines the borders of the organ.

increase in size and a change in shape (Fig. 2). Growth is due, at least in part, to increased cell number and, therefore, can be monitored by measuring the DNA content of the tissue. SVs dissected from the neonate contained ≈1.3 μg of DNA (data not shown). After 4 days in culture, there was a significant increase in DNA content especially in the organs treated with androgen (data not shown), and this pattern was even more pronounced after 6 days in culture (Fig. 3). When the anti-KGF mAb was added to the medium, there was significant reduction in the androgen-stimulated growth observed. Addition of recombinant human KGF at 10 ng/ml to the medium containing anti-KGF plus testosterone resulted in growth equivalent to that obtained in the presence of testosterone alone. The ability of exogenous KGF to overcome the growth inhibitory effect of the antibody further established that the inhibition observed was KGF-specific.

Growth of the SV also could be evaluated by measuring the area of the tissue by digitized image analysis of whole-mount

Table 1. Specificity of neutralizing KGF mAb 2C4

Mitogen	[3H]Thymidine incorporation, cpm	
	− 2C4	+ 2C4
None	336 ± 159	296 ± 112
KGF*	85,783 ± 10,856	3,401 ± 635
aFGF	30,188 ± 3,052	29,570 ± 7004
bFGF	4,597 ± 582	4,718 ± 866
EGF	22,522 ± 2,027	22,614 ± 1459

[3H]Thymidine incorporation into trichloroacetic acid-precipitable material was measured in BALB/MK cells in response to various growth factors in the absence (−) or presence (+) of 2C4 antibody. Data are expressed as the mean ± SD of triplicate measurements. Growth factor concentrations were 10 ng/ml; 2C4 concentration was 6 μg/ml. aFGF, acidic FGF; bFGF, basic FGF.
*Similar results were obtained with mouse KGF partially purified from NIH 3T3 culture fluids.

photographs. By using this approach, it was possible to distinguish between changes in the epithelial and mesenchymal components of the organ. The data presented in Table 3 demonstrate that the androgen-dependent growth of the SV was due primarily to expansion of the epithelial compartment. This was consistent with the epithelial specificity of KGF observed in other contexts (12). When anti-KGF was included with testosterone, the mean epithelial area was significantly reduced, particularly after a 4-day incubation period. The increase in mesenchymal area in all groups that was neither androgen-dependent nor affected by anti-KGF was attributed primarily to insulin present in the basal medium (24).

KGF Can Partially Substitute for Androgen in Supporting SV Growth. Because anti-KGF reduced the growth-promoting effect of androgen on SV in organ culture, we tested whether KGF could replace testosterone in supporting this growth. KGF at 100 ng/ml stimulated an increase in SV DNA content comparable to that elicited by a suboptimal dose of testosterone (10^{-10} M) or ≈50% of the effect obtained with an optimal dose of testosterone (10^{-7} M) (Fig. 4). A lower KGF concentration (20 ng/ml) did not have a statistically significant effect. The stimulatory action of KGF was not augmented by a suboptimal dose of testosterone. Moreover, branching morphogenesis was not induced by exogenous KGF or a suboptimal dose of testosterone, either alone or in combination (data not shown). These findings indicate that while KGF is required for normal androgen-mediated SV branching morphogenesis, it is not sufficient to replace all androgen functions.

DISCUSSION

The present studies establish the role of KGF as a stromally derived effector of androgen action in a neonatal mouse SV organ culture system in which the target tissue undergoes androgen-dependent growth and differentiation in a manner closely resembling the normal pattern *in situ*. A KGF mAb with demonstrated specificity for KGF neutralization dramatically retarded branching morphogenesis in the SV and inhibited SV growth as measured by DNA content. Image analysis of the tissue indicated that this inhibition by KGF mAb was localized to the epithelial compartment without any effect on the mesenchyme, consistent with the known epithelial target cell specificity of KGF (12).

Due to its small size, quantitative analysis of KGF production in the SV of newborn mice was limited. Nonetheless, KGF transcript expression was verified, and the KGF transcript was shown to be inducible by androgen in SV-derived primary mesenchymal cell cultures. It was not possible to propagate neonatal SV epithelial cells in culture. However, a large number of epithelial cells from a wide variety of sources have been found to lack KGF expression (ref. 11 and unpublished observations), and thus, it seems unlikely that SV epithelial cells contribute to KGF production.

The actions of exogenous KGF in organ culture provided further insights into the role of KGF in the developing mouse

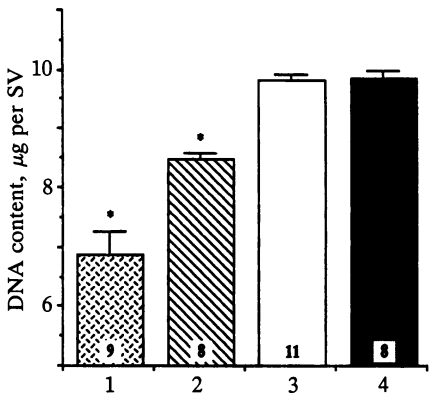

FIG. 3. Effect of anti-KGF of SV growth. Neonatal SVs were dissected and placed in organ culture. They were incubated in a basal medium with the addition of mouse control IgG (bar 1); anti-KGF plus testosterone at 10^{-7} M (bar 2); anti-KGF, KGF at 10 ng/ml, plus testosterone at 10^{-7} M (bar 3); or mouse control IgG plus testosterone at 10^{-7} M (bar 4). Antibodies were utilized at a final concentration of 6 μg/ml. Cultures were incubated for 6 days and assayed for total DNA content. A minimum of eight samples recovered from two or three experiments was assayed. Values represent the DNA content per SV (mean ± SEM). Statistical differences were determined using Student's *t* test for independent samples. There was no statistical difference between bars 3 and 4.
*Bars 2 and 1 were significantly different from bars 3 and 4 ($P \leq 0.001$).

Table 2. Node analysis of branching morphogenesis

Treatment	Day	Node number	*n*
IgG	4	0.2 ± 0.2*	7
IgG + T		10.7 ± 2.4	7
Anti-KGF + T		2.4 ± 0.1*	10
IgG	6	0.3 ± 0.2*	7
IgG + T		42.2 ± 2.0	7
Anti-KGF + T		14.9 ± 1.8*	7

Whole-mount photographs of SVs grown for either 4 or 6 days in medium containing mouse control IgG (6 μg/ml), mouse control IgG plus testosterone (T, 10^{-7} M), or anti-KGF (6 μg/ml) plus testosterone (10^{-7} M) were digitized and the node number per SV was determined. Seven to 10 specimens (*n*) were analyzed from each group. Results represent the mean ± SEM. Statistical differences were determined by Student's *t* test.
*$P \leq 0.001$ relative to mouse control IgG plus testosterone.

Table 3. Effect of anti-KGF treatment on the area of individual tissue layers

Treatment	Day	Area, mm²		*n*
		Epithelium	Mesenchyme	
IgG	4	47.1 ± 4.7*	128.4 ± 12.3	6
IgG + T		145.5 ± 8.2	114.3 ± 11.6	6
Anti-KGF + T		94.4 ± 5.5*	117.4 ± 4.1	10
IgG	6	89.8 ± 8.6*	234.0 ± 15.7†	5
IgG + T		287.2 ± 13.3	175.5 ± 14.7	6
Anti-KGF + T		247.5 ± 8.8†	159.6 ± 7.7	6

Whole-mount photographs of SVs grown for either 4 or 6 days in medium containing mouse control IgG (6 μg/ml), mouse control IgG plus testosterone (T, 10^{-7} M), or anti-KGF (6 μg/ml) plus testosterone (10^{-7} M) were digitized and the area of individual tissue layers was determined. Five to 10 specimens (*n*) were analyzed from each group.
*$P < 0.001$ relative to mouse control IgG plus testosterone.
†$P < 0.05$ relative to mouse control IgG plus testosterone.

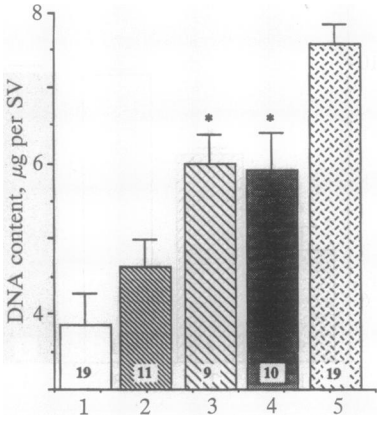

FIG. 4. Effect of exogenous KGF on growth. Intact SVs were grown in medium containing various concentrations of KGF and testosterone. Bars: 1, no addition; 2, KGF at 20 ng/ml; 3, KGF at 100 ng/ml; 4, 10^{-10} M testosterone; 5, 10^{-7} M testosterone. The number of samples in each group is shown at the base of the column and represents samples from two to four experiments. Statistical differences were determined using Student's *t* tests for independent samples. Growth stimulated by KGF at 100 ng/ml and a suboptimal dose of testosterone (10^{-10} M) was significantly higher than basal levels.
*$P < 0.01$.

SV. KGF was shown to partially substitute for testosterone in supporting SV growth. The large dose required is probably due to difficulties associated with penetration of KGF into the SV explant. The fact that KGF, a suboptimal dose of testosterone, or a combination of the two was not sufficient to optimally stimulate branching morphogenesis suggests that other factors must also contribute to testosterone-dependent SV development. By analogous approaches using neutralizing antibodies against other growth factors, it may be possible to identify additional ligands involved in this process.

A striking feature of SV development is the morphogenesis of a distinctive, highly complex, epithelial branching pattern. Regulation of branching morphogenesis by autocrine/paracrine growth factors produced in response to estrogen has been implicated in mammary gland and uterine development (25, 26). While there is a large body of evidence that mesenchyme expressing androgen receptors is required for SV epithelial development (2–4, 7, 8, 13), the agents responsible for this effect were unknown. To our knowledge, our present report is the first to identify a specific growth factor as a mediator of such developmental processes in an androgen-dependent organ system.

Branching morphogenesis involves changes in epithelial cell shape (27), interactions with extracellular matrix (28, 29), and synthesis of matrix-degrading proteases (30) whose expression is regulated by growth factors (31–33) and sex hormones (34–37), in addition to epithelial proliferation. KGF stimulates cell migration and urokinase activity of epidermal keratinocytes (38), consistent with the idea that its action in the SV may involve the expression of differentiated functions along with stimulation of cell division. It will be of interest to determine whether KGF induces the androgen receptor in SV epithelium, a key marker of differentiation in the postnatal period. Recent studies indicate that KGF expression is modulated by androgens in adult prostatic fibroblasts (ref. 39 and unpublished observations). Thus, KGF may play a role as a paracrine mediator in a variety of androgen-dependent organs, both during development and in the adult.

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