Interaction of Androgen-induced Autocrine Heparin-binding Growth Factor with Fibroblast Growth Factor Receptor on Androgen-dependent Shionogi Carcinoma 115 Cells

Norio Nonomura, Jian Lu, Akira Tanaka, Hiroshi Yamanishi, Bunzo Sato, Takao Sonoda, and Keishi Matsumoto

Departments of Pathology [J. L., A. T., H. Y., K. M.], Internal Medicine [B. S.], and Urology [N. N., T. S.], Osaka University Medical School, Kita-ku, Osaka 530, Japan

ABSTRACT

Stimulation of a Shionogi carcinoma 115-derived cultured cell line (SC-3) with androgen resulted in secretion of heparin-binding growth factor. In this study, we analyzed cell-surfaced receptors for growth factors. Binding data of growth factors on intact SC-3 cells revealed the presence of low and high affinity receptors for epidermal growth factor (EGF), insulin, and fibroblast growth factor (FGF). The dissociation constant values were 50 pM and 1.0 nM for EGF, 1.2 nM and 30 nM for insulin, and 34 pM and 7.5 nM for FGF. The numbers of maximal binding sites were 300 and 900/cell for EGF, 2,000 and 14,000/cell for insulin, and 13,000 and 81,000/cell for FGF. To examine the association of androgen-induced growth factor with one of these receptors, the conditioned medium prepared from androgen-stimulated SC-3 cells was fractionated through a heparin-Sepharose column. Growth factor activity adsorbed and eluted by 1 M NaCl from the column was comigrated with the activity inhibiting FGF-receptor association. In addition, basic 125I-FGF was cross-linked, using disuccinimidyl suberate, to the receptor with an apparent molecular weight of 130,000, whose labeling was inhibited when basic FGF, acidic FGF, or highly purified androgen-induced growth factor was present in excess. Furthermore, the highly purified growth factor, basic FGF, or androgen-induced growth of SC-3 cells was significantly and similarly inhibited by anti-basic FGF antibody IgG. These results indicate that androgen-induced FGF-like factor acts as an autocrine growth factor via the FGF receptor in a process of SC-3 cell proliferation.

INTRODUCTION

Sex hormones have been well recognized as mitogens in human prostate and breast carcinomas. The presence of intra-cellular receptors for sex hormones is considered to be a prerequisite for these cells to respond to hormonal stimuli, suggesting that gene products activated by sex hormones play a crucial role in tumor growth (1, 2). The available data have suggested that the growth of transformed target cells is enhanced by an induction of autocrine growth factors (reviewed in Ref. 3). In response to estrogenic stimuli, the human breast cancer cell line, MCF-7, has been reported to secrete various growth-promoting polypeptides such as transforming growth factor-α (4), insulin-like growth factor-1 (5), and a protein with a molecular weight of 52,000 (6). However, it remains to be elucidated which growth factor is responsible for estrogen-responsive growth of MCF-7 cells. For instance, the growth-promoting activity and EGF3 receptor-binding activity in the conditioned medium from estrogen-stimulated MCF-7 cells were coeluted from gel filtration columns (4), but anti-EGF receptor antibody was reported to be able to antagonize transforming growth factor-α-induced, but not estrogen-induced, proliferation of MCF-7 cells (7). These complicated results hinder our ability to establish a unified theory for estrogen-induced growth of MCF-7 cells.

SC 115, originally developed in the mammary glands of a female mouse, has been maintained as an androgen-responsive tumor for more than 20 years in vivo (8, 9). Recently, we established a cultured cell line, termed SC-3, derived from a SC 115 tumor which shows remarkable androgen-dependent growth in a serum-free culture condition (9, 10). Taking advantage of our serum-free culture system, we addressed the possibility that stimulation of SC-3 cells with androgen results in secretion of an autocrine growth factor(s). Our results indicate that androgen-induced secretory growth factor is eluted from a heparin-Sepharose affinity column at 1 M NaCl (11, 12), whose characteristics display some similarities with those of FGFs (13). Actually, basic or acidic bovine FGF has been observed to stimulate the growth of SC-3 cells (14).

In order to demonstrate that androgen-induced growth factor acts on SC-3 cells in an autocrine fashion, SC-3 cells should contain the cell-surfaced receptor for this growth factor. Furthermore, characterization of the cell-surfaced receptor would provide us with the important clue for the molecular nature of this growth factor itself. The data described in this communication indicate that androgen-induced heparin-binding growth factor does interact with the FGF receptor.

MATERIALS AND METHODS

Chemicals. [methyl-3H]Thymidine, EGF, nonradioactive steroids, and heparin-Sepharose were obtained as described previously (10, 12). BSA (essential fatty acid free), DSS, and bovine insulin were purchased from Sigma Chemical Co. (St. Louis, MO). Unlabeled bovine brain aFGF and bFGF were from R&D System Inc. (Minneapolis, MN). 125I-bFGF (1400 Ci/mmol), 125I-EGF (100 μCi/μg), and 125I-insulin (receptor grade; 1000 Ci/mmol) were from Amersham International plc (Buckinghamshire, England). 14C-labeled protein markers for SDS-PAGE were from Amersham. Antibody IgG against bovine brain bFGF (R&D System, Inc.) and control IgG were obtained as described previously (15). The other chemicals were of analytical grade.

Cloning and Cell Culture. The cell lines used in the present study were derived from a SC 115 tumor. The methods for cloning SC-3 cells (one of the androgen-responsive cell lines obtained) and SC-4 cells (one of the androgen-unresponsive cell lines obtained) were described previously (9, 12). SC-3 and SC-4 cells were cultured continuously in a serum-free culture condition (9, 10). Taking advantage of our serum-free culture system, we addressed the possibility that stimulation of SC-3 cells with androgen results in secretion of an autocrine growth factor(s). Our results indicate that androgen-induced secretory growth factor is eluted from a heparin-Sepharose affinity column at 1 M NaCl (11, 12), whose characteristics display some similarities with those of FGFs (13). Actually, basic or acidic bovine FGF has been observed to stimulate the growth of SC-3 cells (14).

Preparation of Serum-free CM and Purification of Androgen-induced Growth Factor. SC-3 cells (104 cells/100-mm dish) were plated and cultured as described previously (12). Serum-free CM (1–2 liters) obtained in the presence of testosterone (10−8 M)-stimulated SC-3 cells was collected, filtered, and concentrated (up to 25-fold) as described previously (12).

The concentrated CM (usually 40–80 ml) was applied to a heparin-Sepharose affinity column at 1 M NaCl (11, 12), whose characteristics display some similarities with those of FGFs (13). Actually, basic or acidic bovine FGF has been observed to stimulate the growth of SC-3 cells (14).

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2 To whom correspondence should be addressed.

3 Abbreviations used here are: EGF, epidermal growth factor; BSA, bovine serum albumin; DSS, disuccinimidyl suberate; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; SC 115, Shionogi carcinoma 115; MEM, Eagle’s minimal essential medium; CM, conditioned medium; PBS, phosphate-buffered saline; CHAPS, 3-(3-cholamidopropyl)dimethylammonio-1-propane sulfonate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Sepharose column (gel bed volume: 1.2 ml) which had been preequilibrated with 10 mM Tris-HCl buffer (pH 7.0 at 20°C) containing 0.1 M NaCl and 0.2% (w/v) CHAPS. After being washed with 100 ml of the equilibrated buffer, materials bound to the column were eluted with a linear gradient of 0.1–2.0 M NaCl in 10 mM Tris-HCl buffer (pH 7.0 at 20°C) containing 0.2% (w/v) CHAPS (30 ml) at a flow rate of 10 ml/h. Each fraction was dialyzed against 2 liters of Ham’s F-12 medium supplemented with 25 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid-0.2% (w/v) gelatin (pH 7.4 at 20°C) at 4°C for 24 h. The aliquots of each fraction were used to measure the growth stimulatory activity for SC-3 cells as published before (10). The fractions showing the activity to enhance the [3H]thymidine incorporation into SC-3 cells were combined and applied to a copper affinity column which had been prepared according to the method of Shing (16). Materials absorbed onto a copper affinity column were eluted with a linear (2–10 mM) imidazole gradient in 10 mM Tris-HCl buffer (pH 7.0 at 20°C) containing 0.1 M NaCl and 0.2% (w/v) of CHAPS. The growth stimulatory activity in each fraction was measured as described above. The active fractions were applied to a second heparin-Sepharose affinity column, and the biological activity in each fraction was measured as described above. Then, samples in each fraction were analyzed by 15% SDS-PAGE as described by Laemmli (17), and the polypeptide bands were visualized by silver staining (18).

Binding of [125I]-bFGF to SC-3 and SC-4 Cells. SC-3 and SC-4 cells were plated on 24-well tissue culture clusters in 1 ml of MEM containing 2% (v/v) dextran-coated charcoal-treated fetal calf serum (no testosterone) at 10^5 and 5 × 10^5 cells/well, respectively. On the following day (day 0), the medium was changed to the serum-free medium (no testosterone). Subconfluent cultures were transferred to 15°C on days 2–4. The binding assay was carried out as described by Neufeld and Gospodarowicz (19) with minor modifications. Briefly, 0.2 ml of a binding buffer composed of Ham’s F-12 medium-25 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (pH 7.4 at 20°C)-0.2% (w/v) gelatin was added to each well after the cells were washed twice with PBS. To obtain the binding parameters, varying concentrations of unlabeled bFGF were added to the binding buffer. Nonspecific binding was determined in the presence of an excess (1 μg/ml) of unlabeled bFGF. Then, [125I]-bFGF was added to each well [80 pg (14,000 dpm)/ml]. The binding of [125I]-bFGF to the cells was permitted by incubation at 15°C for 4 h at a final volume of 0.5 ml unless specified otherwise. After incubations, the cells were washed three times with ice cold PBS supplemented with 0.1% (w/v) BSA at 4°C and then lysed with 0.5 ml of 1% (w/v) Triton X-100-0.1% (w/v) BSA in water for 1 min. The radioactivity of the cell lysates was determined in a Packard MINAXI gamma-counter. The binding data were analyzed according to the Scatchard procedure (20). To examine the binding specificity, SC-3 cells were also incubated with [125I]-bFGF (80 pg/ml) in the presence of unlabeled growth factors or the fractionated CM as described above. Correction with nonspecific binding obtained by incubation with an excess (1 μg/ml) of bFGF was always carried out. All binding assays were performed in duplicate.

Binding of [125I]-EGF or [125I]-Insulin to SC-3 Cells. The binding of [125I]-EGF or [125I]-insulin on SC-3 cells was evaluated by the same method as used for FGF binding except for the binding buffer; the buffer contained 0.1% (w/v) BSA in stead of gelatin.

Cross-Linking of [125I]-bFGF to SC-3 Cells. SC-3 cells were cultured in the serum- and testosterone-free medium in 24-well tissue culture clusters for 2 days. The cells were washed twice with PBS and then incubated in the binding buffer containing [125I]-bFGF (1 ng/ml) in the presence or absence of unlabeled growth factors at 15°C for 4 h. After incubations, the cells were washed as mentioned above, and 0.4 ml of PBS (pH 8.3 at 20°C) containing 1 mM MgCl2 was added. Then, DSS was added to each well at the final concentration of 0.3 mM (21). The reaction, mixtures were tumbled at 4°C for 20 min. The reaction was terminated by adding 0.8 ml of quenching buffer containing 10 mM Tris-HCl (pH 7.5 at 20°C)-0.2 M glycine-1 mM EDTA. The cells were scraped off with a rubber policeman and pelleted down at 400 × g for 3 min. The pellet was lysed in cold lysis buffer [0.3 M NaCl-0.5% (w/v) Nonidet P-40-50 mm Tris, pH 7.4 at 20°C] for 10 min. The suspension was centrifuged at 10,000 × g for 40 min, and aliquots from the supernatant were used for analysis by SDS-PAGE after being boiled for 5 min in electrophoretic sample buffer (17). The samples were electrophoresed with 7.5% SDS-PAGE under the reduced condition. The gels were fixed and then dried and autoradiographed at −70°C using Kodak X-Omat R film and Dupont Lighting Plus intensifying screens.

In some experiments, SC-3 cells briefly (1–2 min) digested with 0.01% (w/v) trypsin-0.02% (w/v) EDTA were used for the cross-linking experiments. In this case, the suspended cells (8 × 10^6 cells/0.5 ml/tube) were incubated and cross-linked with [125I]-bFGF as described above.

Effect of Anti-bFGF Antibody IgG on DNA Synthesis in SC-3 Cells. The method was described previously (15).

RESULTS

Binding Characteristics of Cell-surfaced Receptors on SC-3 and SC-4 Cells. In view of our previous observations that FGFs (14), but neither EGF nor insulin (14), stimulated growth of SC-3 cells, the presence of the cell-surfaced receptors for these growth factors on SC-3 cells was examined. As shown in Fig. 1, when binding was analyzed as a function of unlabeled FGF concentrations, the ability of increasing concentrations of unlabeled bFGF to displace [125I]-bFGF bound to SC-3 cells was demonstrated. aFGF had a similar ability but it was less. Scatchard plot analysis on the binding data of bFGF indicates the presence of the high and low affinity binding sites (Fig. 2).

Characterization of FGF Receptors on SC-3 Cells. When [125I]-bFGF (80 pg/ml) ± unlabeled bFGF (1 μg/ml) was incubated with SC-3 cells at various temperatures (4, 15, and 37°C), apparent saturation of [125I]-bFGF binding toward the high affinity site was achieved by 4 h incubation at 4–15°C. After the association reached a plateau at these temperatures, the amount of [125I]-bFGF bound to the cells was not significantly diminished up to 7 h (data not shown). Affinity cross-linking of [125I]-bFGF
to cell-surfaced receptors was also done to further characterize the binding sites. When intact SC-3 cells attached on the dish were incubated with $^{125}$I-bFGF (1 ng/ml) at 15°C for 4 h and then subjected to cross-linking with DSS as described in "Materials and Methods," the specifically labeled band migrated on electrophoresis at positions corresponding approximately to $M_r$ ~150,000. Assuming that the molecular weight includes that of $^{125}$I-bFGF, the apparent molecular weight of FGF receptor can be calculated as 130,000. This band was almost completely abolished when SC-3 cells were incubated with $^{125}$I-bFGF in the presence of an excess (1 $\mu$g/ml) of unlabeled bFGF or aFGF (data not shown).

Interaction of Androgen-induced Heparin-binding Growth Factor with FGF Receptor. Since androgen-induced growth factor secreted from SC-3 cells has been characterized as FGF-related polypeptide (11, 12), we examined the possibility that this growth factor is associated with the FGF receptor identified on SC-3 cells. In the first step, the concentrated CM was applied to a column of heparin-Sepharose and eluted with a linear NaCl gradient (Fig. 3). One peak of growth factor activity, as measured by the ability of each fraction to stimulate DNA synthesis in SC-3 cells, was observed at 1.0–1.2 M NaCl; a peak of the growth stimulatory activity induced by native bFGF was found at 1.5 M NaCl (data not shown). The ability of each fraction to displace $^{125}$I-bFGF bound to SC-3 cells was also examined. Two peaks were identified. The first and second peaks eluted at 0.5 M and 1.1 M NaCl, respectively. Although the biological significance of the first peak remained obscure, comigration of growth factor activity of the second peak with the ability to displace bound $^{125}$I-bFGF would suggest that androgen-induced heparin-binding growth factor is able to bind to the FGF receptor. To confirm this, a highly purified preparation was used. For this purpose, this growth factor was purified by a three-step procedure. The growth factor partially purified by the first heparin-Sepharose column was applied to a copper affinity column. The growth-promoting activity was found to be eluted at 8 mM of imidazole (Fig. 4). The active fractions were then applied to the second heparin-Sepharose column (Fig. 5).

Analysis of the active fractions from the second column by SDS-PAGE and silver staining revealed the presence of two bands with molecular weights of 16,000 and 18,000. The intensities of both bands were in parallel with the biological activity in each fraction. Using this highly purified growth factor, its ability to displace bound of $^{125}$I-bFGF was addressed by cross-linking procedure. In this particular experiment, the cell suspension obtained by brief trypsinization was incubated with $^{125}$I-bFGF in the absence or presence of the highly purified growth factors, after trypsin activity was completely eliminated by trypsin inhibitor. As shown in Fig. 6, the cross-linked bands corresponding at $M_r$ ~150,000 were observed on autoradiography, which was similar to the result described above for intact SC-3 cells attached on the dish. These results also indicate that the brief pretreatment of SC-3 cells with trypsin does not interfere with cross-linking experiments. Since more quantitative and effective labeling was achieved by using the cell suspension, the highly purified growth factor was incubated with pretrypsinized cells. As shown at Lane 5 in Fig. 6, the cross-linked bands observed at Lane 5 in Fig. 6, the androgen-induced growth factor effectively inhibited the formation of cross-linked $^{125}$I-bFGF-receptor complexes. In contrast, the materials in the fraction lacking the growth-promoting ability failed to inhibit the formation of $^{125}$I-bFGF-receptor complexes (Lane 4 in Fig. 6). A similar observation was obtained by using SC-3 cells attached onto dishes (data not shown). Antibody IgG against bFGF was also found to significantly decrease the growth of SC-3 cells induced by the same highly purified growth factor similar to that induced by bFGF or testosterone (Table 2).

**DISCUSSION**

The present studies clearly demonstrated that SC-3 cells contain the receptors for EGF, insulin, and FGF, although the

![Graph](image)

**Fig. 2. Scatchard analysis of the data on the specific binding of $^{125}$I-bFGF to SC-3 cells. SC-3 cells were incubated with $^{125}$I-bFGF (80 pg/ml) in the presence of various concentrations of unlabeled bFGF as described in "Materials and Methods." The specifically labeled band migrated on electrophoresis at positions corresponding approximately to $M_r$ ~150,000. Assuming that the molecular weight includes that of $^{125}$I-bFGF, the apparent molecular weight of FGF receptor can be calculated as 130,000. This band was almost completely abolished when SC-3 cells were incubated with $^{125}$I-bFGF in the presence of an excess (1 $\mu$g/ml) of unlabeled bFGF or aFGF (data not shown).**

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**Table 1 Binding parameters of cell-surfaced receptor**

<table>
<thead>
<tr>
<th>Ligands</th>
<th>$K_d$</th>
<th>Maximal binding sites/cell</th>
<th>$K_d$</th>
<th>Maximal binding sites/cell</th>
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<tr>
<td>SC-3 cells</td>
<td></td>
<td></td>
<td>SC-4 cells</td>
<td></td>
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<tr>
<td><strong>Ligands</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FGF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High affinity sites</td>
<td>$34.0 \pm 9.0^{a,b}$</td>
<td>$13,000 \pm 1,000$ ($n = 3$)</td>
<td>$13$</td>
<td>$17,000$ ($n = 1$)</td>
</tr>
<tr>
<td>Low affinity sites</td>
<td>$7.5 \pm 2.9$</td>
<td>$810,000 \pm 200,000$ ($n = 3$)</td>
<td>$4.6$</td>
<td>$3,700,000$ ($n = 1$)</td>
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<tr>
<td>EGF</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>High affinity sites</td>
<td>$50$</td>
<td>$300$ ($n = 2$)</td>
<td></td>
<td></td>
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<tr>
<td>Low affinity sites</td>
<td>$1.0$</td>
<td>$900$ ($n = 2$)</td>
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<tr>
<td>Insulin</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>High affinity sites</td>
<td>$1.2$</td>
<td>$2,000$ ($n = 2$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low affinity sites</td>
<td>$30$</td>
<td>$14,000$ ($n = 2$)</td>
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</table>

* $^{a}$ Mean ± SE.
* $^{b}$ n, number of experiments; ND, not determined.
Fig. 3. Heparin-Sepharose chromatographic patterns of growth-promoting activity and FGF receptor-associable ability present in CM from androgen-stimulated SC-3 cells. The concentrated CM from androgen-stimulated SC-3 cells was fractionated by a heparin-Sepharose column. The growth-promoting activity in each fraction was assayed using SC-3 cells as target cells. Another aliquot was incubated with SC-3 cells in the presence of \(^{125}\)I-bFGF to quantify the ability to displace the binding of \(^{125}\)I-bFGF to SC-3 cells.

Fig. 4. Copper affinity chromatography of autocrine growth factor secreted from androgen-stimulated SC-3 cells. The autocrine growth factor partially purified by a heparin-Sepharose column was applied to a copper affinity column and eluted by a linear imidazole gradient. The aliquot in each fraction was used for quantifying the growth-promoting ability for SC-3 cells as described in “Materials and Methods.”

Fig. 5. Purification of androgen-induced autocrine growth factor by three-step method. The androgen-induced autocrine growth factor partially purified by a heparin-Sepharose column and copper affinity column was further fractionated by a second heparin-Sepharose column (top). The active fractions (fraction numbers from 16–26) were analyzed by SDS-PAGE. The proteins present in each fraction were visualized by the silver staining method. As a control, bFGF (1 ng/lane) was also electrophoresed. The protein markers used in this experiment (Lane M) were ovotransferrin (M, 78,000), chick serum albumin (M, 66,000), ovalbumin (M, 49,000), bovine myoglobin (M, 17,200), and bovine cytochrome c (M, 12,300).

Fig. 6. Inhibition of cross-linking between \(^{125}\)I-bFGF and its cell surface receptor by highly purified androgen-induced growth factor. SC-3 cells were briefly treated by trypsin whose activity was then inhibited by trypsin inhibitor as described in “Materials and Methods.” The aliquots of the cell suspension were incubated with \(^{125}\)I-bFGF (1 ng/ml alone (Lane 1) or in the presence of unlabeled aFGF (1 ng/ml) or bFGF (1 ng/ml) (Lane 2). The androgen-induced growth factor (0.1 \(\mu\)g/ml as bFGF bioactivity) was also used as a control (Lane 4). These samples were subjected to the cross-linking reaction, after the incubated cells were washed. Equivalent amount of protein (50 \(\mu\)g) was loaded into each lane for SDS-PAGE analysis. As a control, an equal volume of the eluate at fraction 2 from the first heparin-Sepharose column (no growth-promoting activity) was also used (Lane 5). The protein markers used for SDS-PAGE analysis were myosin (M, 200,000) and phosphorylase b (M, 92,500).
that on SC-3 cells (19). Although the biological role of the low affinity site on SC-3 cells remains to be elucidated, it can be reasonably speculated that the high affinity site is able to transmit FGF signals into target cells. Moreover, it is concluded that SC-3 cells have putative FGF receptors in view of their binding specificity as well as molecular weights (19, 25).

In order to establish an autocrine mechanism in the growth of transformed cells, identification of autocrine growth factor(s) and its receptor would be obligatory. Our previous (11, 12) as well as present studies showed that CM prepared from androgen-stimulated SC-3 cells contains only one peak of the growth factor activity on various column chromatographies (more detailed characterization will be submitted elsewhere). The growth factor activity in CM is really induced by androgen and not a BSA contaminant, androgen-independent factor, or androgen itself. This conclusion has been obtained by the following findings: (a) the growth factor activity was found in serum-free CM obtained in the presence, but not in the absence, of androgen (12); (b) cyproterone acetate almost completely inhibited androgen-induced but not at all the growth factor-induced growth of SC-3 cells (11, 12); (c) the growth factor activity was markedly inactivated by trypsin or heating at 100°C (11); (d) the recovery of the growth factor activity in active fractions from each column (70–80%) was very much higher than that of testosterone (11, present study); and (e) androgen induced the growth factor activity from SC-3 cells even in a protein-free medium [Ham’s F-12:MEM (1:1, v/v)]. The present study demonstrated that the purified androgen-induced growth factor can be associated with FGF receptor on SC-3 cells, strongly suggesting that this growth factor is an FGF-like polypeptide.

This is further supported by our recent and present findings that the growth of SC-3 cells induced by androgen, bFGF, or this purified androgen-induced growth factor is significantly and similarly inhibited by antil-bFGF antibody IgG (Ref. 15; Table 2). Furthermore, the androgen-induced growth factor eluted from a heparin-Sepharose column cross-reacted with anti-bFGF antibody when examined by radioimmunoassay; the cross-reactivity was weak but was exactly in parallel to the growth stimulatory activity (26). However, Western blot analysis using anti-bFGF antibodies detected definite bands for the purified androgen-induced growth factor-stimulated [3H]thymidine uptake in SC-3 cells by anti-bFGF antibody IgG or control IgG. On day 3, [3H]thymidine uptake in the SC-3 cells was estimated (15).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[3H]Thymidine uptake (dpm/well)</th>
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<tbody>
<tr>
<td></td>
<td>Antibody IgG</td>
</tr>
<tr>
<td>None</td>
<td>140 ± 11.2*</td>
</tr>
<tr>
<td>10^-4 M testosterone</td>
<td>23,900 ± 1,570*</td>
</tr>
<tr>
<td>1 ng/ml bFGF</td>
<td>2,860 ± 298*</td>
</tr>
<tr>
<td>Purified androgen-induced growth factor</td>
<td>18,000 ± 1,140*</td>
</tr>
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* Mean ± SE of three determinations.

REFERENCES


11. Nakamura, N., Yamanishi, H., Lu, J., Uchida, N., Nonomura, N., Matsu-


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