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Progression of Androgen-sensitive Mouse Tumor (Shionogi Carcinoma 115) to Androgen-insensitive Tumor after Long-term Removal of Testosterone

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Shionogi Carcinoma 115 (SC115) is an androgen-sensitive transplantable mouse tumor. To study the mode of progression from androgen-sensitive to -insensitive tumor, cloned SC115 cells were serially cultured without androgen. Shortly after withdrawal of androgen, SC115 cells showed markedly decreased growth, but growth resumed gradually with loss of response to androgen and the cells 60 weeks after androgen removal [A(−)60 cells] grew faster than SC115 cells cultured in the presence of androgen. A(−)60 cells showed malignant phenotype with morphological changes and tumorigenicity in male and female mice. Although mRNA and binding capacity of androgen receptor were maintained, the cells after removal of androgen rapidly lost expression of mouse mammary tumor virus-related gene and the loss was irreversible in A(−)60 cells. The stimulating effect of basic fibroblast growth factor (bFGF) temporarily decreased, then recovered to the initial level after long-term androgen removal. This fluctuation of response to bFGF was accompanied with changes in the number of bFGF receptors and amount of bFGF-like substance(s) secreted. The substance(s) seemed to be an FGF-like growth factor different from known factors. It was concluded that progression of SC115 cells to androgen-insensitive ones under an androgen-deprived condition proceeded with adaptation by means of increases in production of an FGF-like growth factor and in binding capacity to this factor.

Key words: Shionogi Carcinoma 115 — Androgen-insensitive tumor — FGF-like growth factor — Tumor progression

It has generally been accepted that hormone-sensitive human tumors, such as prostate and breast cancer, gradually lose hormone-sensitivity and finally progress to autonomous tumors. Many investigators have studied the mode of progression and escape from sensitivity to hormones.[1-4] Adaptation and clonal selection with genetic instability are assumed to play an important role in the processes of progression.[5] However, our understanding is still incomplete, and the use of suitable models should help elucidate the course during progression.

SC115[2] cell line is a spontaneously developed mouse mammary tumor established in 1964.[6] SC115 cells have been widely used as a model of androgen-sensitive tumor, since the mode of androgen sensitivity is well characterized under experimental conditions.[7] SC115 cells contain MMTV sequence in genomic DNA and express MMTV-related mRNA under testosterone stimulation,[8] expression of which is considered one of the parameters of androgen response. It was reported that, shortly after withdrawal of testosterone, SC115 cells change to androgen-insensitive cells wothout expression of MMTV-related mRNA.[9]

To clarify the process of progression to acquire androgen-insensitivity, the present study was extended to examine changes of SC115 cells after long-term removal of androgen.

MATERIALS AND METHODS

Chemicals [^{125}I]bFGF (human, 959 Ci/mmol) was purchased from Amersham International plc (Buckinghamshire, UK). [^{3}H]methyltrienolone (R1881, 86.0 Ci/mmol), unlabeled R1881 and [α-^{32}P]dCTP (3000 Ci/mmol) were obtained from Du Pont/NEN (Boston, USA). Anti-bovine bFGF antibody IgG (rabbit) and bFGF were obtained from R&D Systems (Minneapolis, USA). Preimmune rabbit IgG was purchased from DAKO (Glostrup, Denmark). [^{3}H]Thymidine (51 Ci/mmol), heparin-Ultrogel and molecular weight markers were from the same sources as cited in the previous report.[10]

Cells SC115 tumor was donated by Shionogi Laboratory in 1978, and since then has been maintained in our laboratory. Cloning of SC115 was performed as described previously[11] and cloned cells were designated SC115 cells. SC115 cells have been maintained in minimum essential medium/Ham′ F-12 (1:1, v:v) containing 10% FBS treated with 0.05% dextran-coated 0.5% char-

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[2] The abbreviations used are: SC115, Shionogi Carcinoma 115; (b)FGF, (basic) fibroblast growth factor; CS 2, Chiba subline 2; LTR, long terminal repeat; MMTV, mouse mammary tumor virus; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; FBS, fetal bovine serum; AIGF, androgen-induced growth factor.

coal (maintenance medium) supplemented with 10 n*M* testosterone.

In order to obtain androgen-insensitive cells from SC115 cells, SC115 cells were cultured serially in maintenance medium without testosterone. The cells cultured for X weeks were designated A(−)X cells. The cells were harvested and plated weekly for the next generation. A part of the cells was stored in liquid nitrogen and thawed when required.

In some experiments, CS 2 cell line, an androgen-insensitive subline[10,11] derived from SC115, was used for comparison.

Estimation of cell growth Two$\times 10^4$ cells were plated onto a 35-mm dish containing 2 ml of maintenance medium supplemented with or without 10 n*M* testosterone. After 2 days' culture, the medium was changed to serum-free medium (maintenance medium without FBS) with or without 10 n*M* testosterone, and thereafter the medium was renewed every other day. The number of viable cells was counted by trypan blue exclusion using a hemocytometer.

[^{3}H]Thymidine uptake One$\times 10^4$ cells/well were cultured in 0.5 ml of maintenance medium with [SC115 cells] or without [A(−) cells] 10 n*M* testosterone for 2 days. The medium was replaced with serum-free medium and renewed every other day. To estimate the effect of growth-promoting substances except testosterone, the substances were added to the wells 4 days after the change to serum-free medium, then cells were incubated for 16 h. [^{3}H]Thymidine (0.5 μCi) was added to each well and cells were cultured for 3 h, then radioactivity in the acid-insoluble fraction was counted. When the effect of testosterone was examined, 10 n*M* testosterone was added to the medium throughout the period of culture.

Tumorigenicity DD/S mice obtained from Aburabi Farms (Shiga) were used at 8 weeks of age as recipients. Cells (1.2×10^6) suspended in 0.1 ml of maintenance medium with [SC115 cells] or without [A(−)60 cells] 10 n*M* testosterone were implanted subcutaneously at the dorsomedian region of the neck of recipient mice under ether anesthesia. Four weeks after implantation, the size of the tumor was measured and tumors more than 6 mm in diameter were judged as positive. Tumor diameter was calculated as (width+length)/2.

Preparation of conditioned medium The conditioned medium from SC115 cells or A(−) cells cultured in serum-free medium with [SC115 cells] or without [A(−) cells] 10 n*M* testosterone was filtered, concentrated, and dialyzed.[10] For conditioned medium from SC115 cells, testosterone was removed by dextran-coated charcoal treatment after concentration. The growth-promoting activity in these samples was measured in terms of [^{3}H]thymidine uptake.

Heparin-affinity chromatography The conditioned medium from A(−)60 cells was dialyzed, then applied to a heparin-Ultrogel column (1.6 cm^2 × 20 cm). The column was eluted with a linear gradient of 0.2–3.0 *M* NaCl in 10 m*M* Tris-HCl buffer (pH 7.5) containing 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). Each fraction of 3 ml was separated and growth-promoting activity was determined in terms of [^{3}H]thymidine uptake in SC115 cells.

Inhibition of growth-promoting activity by anti-bFGF antibody Fractions containing growth-promoting substance or bFGF were incubated with various amounts of anti-bFGF antibody at 37°C for 1 h. Preimmune rabbit IgG was used as the control. After incubation, the mixture was centrifuged and the supernatant was assayed for the activity in terms of [^{3}H]thymidine uptake in SC115 cells.

SDS-PAGE An aliquot of sample was denatured and subjected to electrophoresis on slab gels according to the method of Laemmli.[12] The gels were stained using a Bio-Rad silver nitrate stain kit.

Binding of androgen and bFGF One$\times 10^6$ cells were suspended in 0.4 ml of serum-free medium containing 0.1% bovine serum albumin supplemented with various concentrations (0.25 to 5 n*M*) of [^{3}H]R1881 and 1 μM triamcinolone acetonide, then incubated at 37°C for 2 h. Cells were obtained by centrifugation, washed and lysed with 1 *N* NaOH. Radioactivity in the lysate was counted as total binding. Nonspecific binding was estimated in a parallel incubation in the presence of 100-fold excess of unlabeled R1881. Specific binding was calculated by subtracting nonspecific binding from total binding.

Five$\times 10^4$ cells were seeded in wells of a 24-well plate containing 0.5 ml of maintenance medium with [SC115 cells] or without [A(−) cells] 10 n*M* testosterone and cultured for 2 days. The medium was changed to serum-free medium containing 0.15% gelatin for 2 h. Cells were washed with 0.3 ml of serum-free medium containing 25 m*M* N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES, pH 7.5) and 0.15% gelatin, placed in the same medium supplemented with various concentrations of [^{125}I]bFGF (0.125 to 4 ng/ml), then incubated at 4°C for 2 h under gentle shaking.[13] In parallel, 100-fold excess of unlabeled bFGF was added to a culture to obtain nonspecific binding. Cells were washed and treated with 2 *M* NaCl in 20 m*M* sodium acetate buffer (pH 4.0), then radioactivity in the lysate was counted.

The dissociation constant (K_d) and maximum binding sites (B_{max}) were calculated by Scatchard analysis.[14]

Northern blot analysis Total RNA was prepared by the acid guanidinium thiocyanate-phenol-chloroform extraction method.[15] Poly (A)$^+$ RNA was separated using Oligotex-dT 30 (Takara, Kyoto). The following probes

were used: a 1.0 kb pair *Xho*I fragment of AIGF,[16] a 0.7 kb *Eco*RI-*Hin*dIII fragment of human androgen receptor,[17] a 1.8 kb pair *Eco*RI fragment of *hst-1* gene,[18] a 0.6 kb *Sac*I-*Eco*RI fragment of *int-2* gene,[19] and a 1.4 kb pair *Pst*I fragment of MMTV containing LTR region.[20] These probes were labeled with [α-^{32}P]dCTP using a nick translation kit (Takara). Hybridization was performed as described previously.[10]

RESULTS

Growth of SC115 cells after removal of testosterone SC115 cells were serially cultured in maintenance medium without testosterone and various generations of A(−) cells were obtained. Without testosterone, SC115 cells were incapable of growing in serum-free medium, showing androgen-dependency (Fig. 1), but addition of FBS supported the growth. After A(−)30, growth resumed at a similar rate (in both the presence and absence of testosterone) to that of SC115 cells cultured with testosterone. Thereafter A(−) cells showed rapid growth with androgen-insensitivity. SC115 cells showed a spindle appearance, resembling fibroblasts, in the presence to testosterone, but long-term removal of testosterone resulted in polygonal form regardless of the presence or absence to testosterone. A(−)60 cells were used as androgen-insensitive cells in the subsequent experiments.

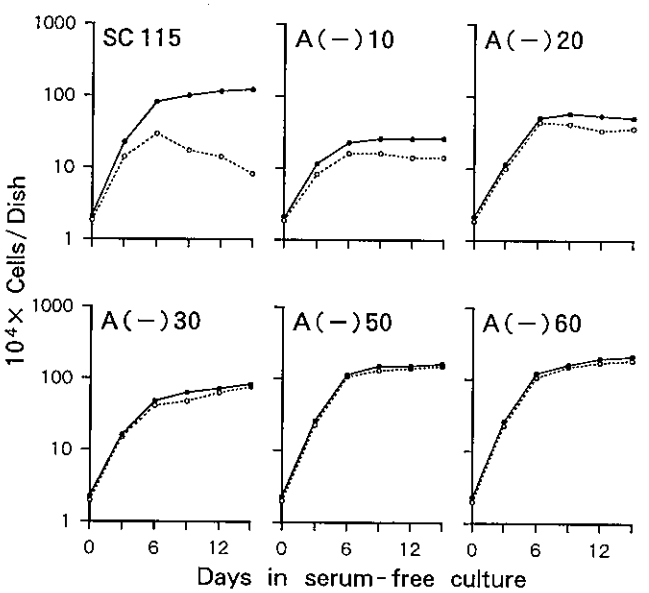

Fig. 1. Growth of SC115 cells and A(−) cells in the presence and absence of testosterone. Cells were cultured with (solid line) or without (dotted line) 10 n*M* testosterone. Data are shown as mean values from three dishes and SE was smaller than the symbols.

Tumorigenicity Implantation of A(−)60 cells evoked tumors at high rate in both male and female mice, while no tumorigenicity was observed with SC115 cells (Table I). Histologically, A(−)60 cells in a tumor were large and spindle-shaped, being different from the small and round SC115 cells in tumors of male mice (Fig. 2).

Effects of testosterone and bFGF on [^{3}H]thymidine uptake of SC115 and A(−) cells [^{3}H]Thymidine uptake of SC115 cells was stimulated 9- and 16-fold by addition of testosterone or bFGF, respectively (Table II). Androgen-stimulated SC115 cells showed high [^{3}H]thymidine uptake without extremely fast growth (Fig. 1), and this may be attributable to the balance of cell division and degradation, since the cells piled up and floated into the medium. Shortly after removal of testosterone, [^{3}H]-thymidine uptake decreased remarkably, and thereafter gradually increased, a similar tendency to that of growth (Fig. 1). The stimulating effect of testosterone was lost rapidly after removal of testosterone, and the unresponsiveness continued. On the other hand, the effect of

Table I. Tumorigenicity of SC115 Cells and A(−)60 Cells

Cells	Recipient mice with tumor	
	Male	Female
SC115	0/18 (0)[a]	0/18 (0)
A(−)60	15/19 (79)	18/20 (90)

a) Percentage of tumor-bearing mice.
Four weeks after transplantation, tumors more than 6 mm in diameter were judged as indicating positive tumorigenicity.

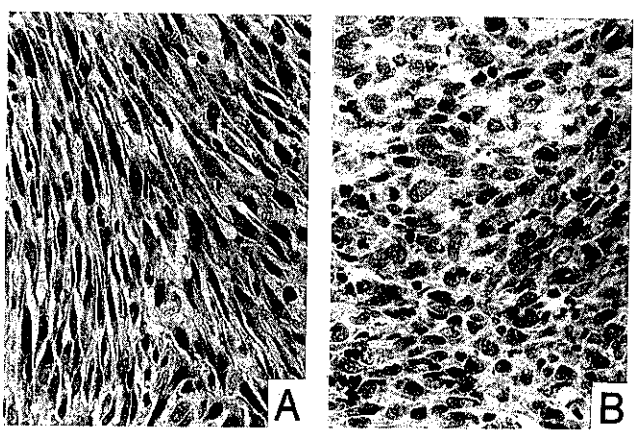

Fig. 2. Microscopic appearance of A(−)60 cells transplanted into mice. A tumor was removed 4 weeks after transplantation of A(−)60 cells into a male mouse. For comparison, SC115 tumor from a male DD/S mouse is shown, since SC115 cells exhibit no tumorigenicity. A; Tumor of A(−)60 cells. B; SC115 tumor. HE (×100).

Table II. Effect of Testosterone and bFGF on [^{3}H]Thymidine Uptake of SC115 and A(−) Cells

Cells	Stimulus		
	Non-stimulated	Testosterone (10 n*M*)	bFGF (1 ng/ml)
SC115	4,028±177	36,305±2,150 (9.0)[a]	64,500±4,139 (16.0)
A(−)1	1,752±108	3,330±205 (1.9)	14,264±1,028 (8.1)
A(−)3	566±49	880±61 (1.6)	1,638±132 (2.9)
A(−)5	1,250±99	924±85 (0.7)	5,586±385 (4.5)
A(−)10	1,877±124	1,729±119 (0.9)	9,003±1,008 (4.8)
A(−)15	1,996±169	1,936±128 (1.0)	10,560±996 (5.3)
A(−)20	2,771±177	2,682±147 (1.0)	14,211±1,019 (5.1)
A(−)30	3,525±293	3,531±266 (1.0)	30,426±2,410 (8.6)
A(−)40	5,202±358	4,988±315 (1.0)	43,456±2,983 (8.4)
A(−)50	4,980±404	4,942±462 (1.0)	71,014±5,201 (14.3)
A(−)60	5,115±381	5,001±379 (1.0)	99,743±6,124 (19.5)

a) Numbers in parentheses are the ratio to the value of non-stimulated cells.
Data are shown as mean±SE (dpm) from three wells.

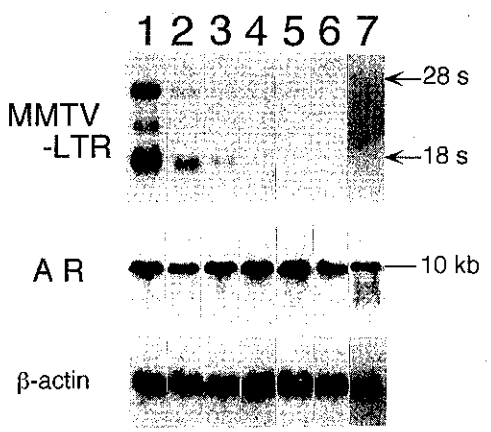

Fig. 3. Northern blot analysis of androgen receptor and MMTV-related mRNA. Poly (A)$^+$ RNAs (3 μg) extracted from SC115 (lane 1), A(−)3 (lane 2), A(−)5 (lane 3), A(−)10 (lane 4), A(−)40 (lane 5), A(−)60 (lane 6) and A(−)60 cells further cultured with testosterone for 12 weeks (lane 7), were electrophoresed and hybridized with probes of androgen receptor (AR), MMTV-LTR and β-actin using the same filter. kb, kilobases; black arrows, sites of ribosomal RNA.

bFGF was reduced shortly after testosterone removal, but then gradually recovered to the level of SC115 cells. Response to bFGF after A(−)50 cells was greater than that in SC115 cells.

Expression of androgen receptor and MMTV-related mRNAs Expression of androgen receptor mRNA was similarly retained among these A(−) cells in spite of loss of sensitivity to testosterone (Fig. 3). MMTV-related mRNA was strongly expressed in SC115 cells, but gradually decreased after removal of testosterone and completely disappeared after A(−)10. A(−)60 cells did not recover expression of MMTV-related mRNA in spite of addition of testosterone for 12 weeks.

Table III. Scatchard Analysis of Androgen and bFGF Binding in SC115 and A(−) Cells

Cells	Androgen receptor		bFGF receptor	
	B_{max}[a]	K_d[b] (n*M*)	B_{max}	K_d (p*M*)
SC115	11.3±0.9	0.43±0.06	20.4±1.4	16.9±1.6
A(−)1	8.6±0.8[e]	0.47±0.04	ND[c]	ND
A(−)3	6.8±0.5[e]	0.57±0.14	10.3±1.0[e]	19.7±1.4
A(−)5	6.6±0.5[e]	0.60±0.11	ND	ND
A(−)10	9.5±0.7[d]	0.38±0.04	12.7±0.8[d]	18.1±1.8
A(−)40	9.4±0.6[d]	0.44±0.05	17.7±1.3	18.1±1.4
A(−)60	9.2±0.6[d]	0.40±0.07	21.8±1.2	16.5±0.9

a) B_{max}, maximum number of binding sites ($\times 10^3$ sites/cells).
b) K_d, dissociation constant.
c) ND, not determined.
d) $P<0.05$, *e*) $P<0.01$; against SC115 cells.
Deta are shown as mean±SE based on three experiments performed independently.

Binding of androgen and bFGF Removal of androgen reduced the number of androgen-binding sites, but the number remained more or less constant among A(−) cells thereafter (Table III). The number of bFGF binding sites declined shortly after removal of testosterone, but thereafter gradually increased and after A(−)50, became greater than in SC115 cells. Affinity of androgen and bFGF binding did not change remarkably in these cells.

Properties of growth-promoting activity in conditioned medium from A(−) cells The growth-promoting activity of conditioned medium obtained from SC115 cells and various generations of A(−) cells was examined in terms of [^{3}H]thymidine uptake in SC115 cells (Fig. 4). Activity in conditioned medium from SC115 cells was remark-

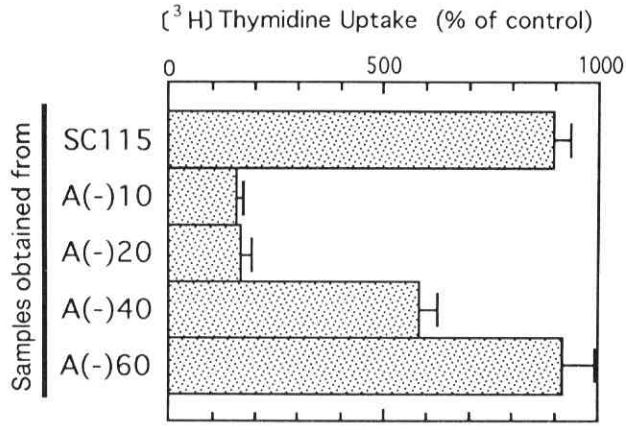

Fig. 4. Growth-promoting activity of conditioned medium obtained from SC115 cells and A(−) cells. Dialyzed conditioned medium was obtained from SC115, and A(−)10, 20, 40, and 60 cells. Samples (10 μg protein) were examined for activity in terms of [^{3}H]thymidine uptake by SC115 cells. Mean ± SE (dpm) were calculated from three independent experiments and data are shown as percentage values with respect to non-stimulated SC115 cells (4085 ± 195 dpm).

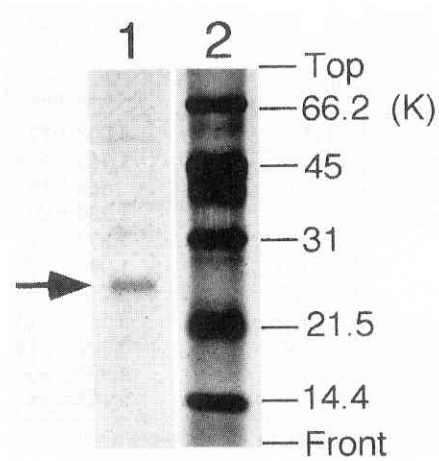

Fig. 6. SDS-PAGE of growth-promoting substance from A(−)60 cells. Twenty μl (8.6 μg as protein) of fraction No. 50 eluted at 0.9 *M* NaCl (Fig. 5) was loaded on 15% polyacrylamide gel (lane 1). Molecular weight markers (lane 2) were bovine serum albumin (Mr 66,200), ovalbumin (Mr 45,000), carbonic anhydrase (Mr 31,000), soybean trypsin inhibitor (Mr 21,500), and lysozyme (Mr 14,400). The black arrow shows the region of Mr 25,000.

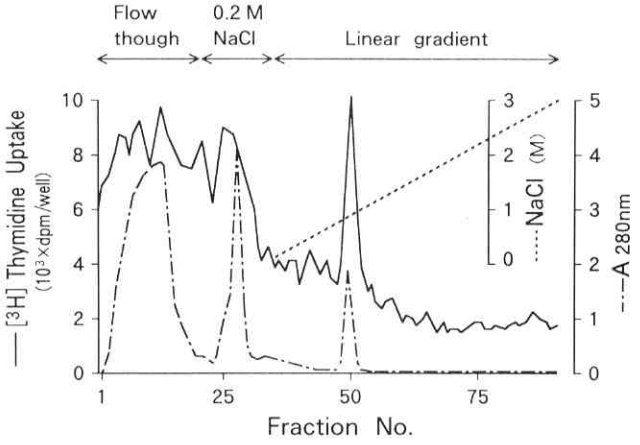

Fig. 5. Heparin-Ultrogel chromatography of dialyzed conditioned medium from A(−)60 cells. Approximately 60 μg (as protein) of dialyzed conditioned medium from A(−)60 cells was applied to a heparin-Ultrogel column and eluted with a linear gradient of 0.2–3.0 *M* NaCl. An aliquot of each fraction was examined for effect on [^{3}H]thymidine uptake by SC115 cells. Protein was measured by UV absorption measurement at 280 nm.

ably high but that from A(−)10 and 20 cells decreased, then gradually recovered with increasing number of generations, and that from A(−)60 cells was as potent as that from SC115 cells. Conditioned medium from A(−)60 cells was dialyzed and applied to a heparin-affinity column. Three peaks showing growth-promoting activity on SC115 cells were obtained; flow-through, 0.2 *M* NaCl eluate, and 0.9 *M* NaCl eluate (Fig. 5). Since the highest specific activity was found in fraction No. 50 eluted with 0.9 *M* NaCl, the growth-promoting substance(s) was assumed to have a heparin-binding nature. An aliquot of fraction No. 50 was subjected to SDS-PAGE (Fig. 6). The main band was located at a position corresponding to approximately Mr. 25,000. The gel corresponding to the main band was sliced and the substance(s) was eluted; growth-promoting activity was confirmed on SC115 cells. Moreover, fraction No. 50 showed dose-dependent growth-promoting activity on A(−)60 cells (Fig. 7). The effect of anti-bFGF on the growth-promoting activity of fraction No. 50 was examined. The stimulating effect of bFGF was completely blocked by anti-bFGF antibody, while the activity of fraction No. 50 was reduced by up to approximately 80% (Fig. 8).

Expression of AIGF and *hst-1* mRNA It was reported that SC115 cells expressed AIGF mRNA under testosterone stimulation.[16] There was no expression of AIGF mRNA in A(−)60 cells or in these cells cultured further with 10 n*M* testosterone for 12 weeks (Fig. 9). CS 2 cells newly showed expression of *hst-1* mRNA,[21] but A(−)60 cells did not express *hst-1* mRNA. The level of *int-2* in A(−)60 cells was also examined and there was no change compared to that in SC115 cells (data not shown).

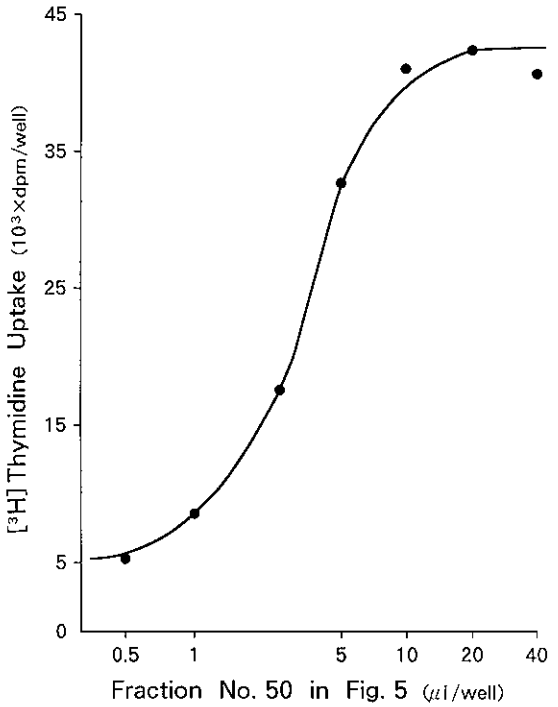

Fig. 7. Autocrine activity of growth-promoting substance on A(−)60 cells. A sample of fraction No. 50 in Fig. 5 (0.43 μg as protein/μl) was added to A(−)60 cells and the growth-promoting activity was measured in terms of [^{3}H]thymidine uptake. Each point was based on three wells. Data are shown as mean and SE lies within the symbols.

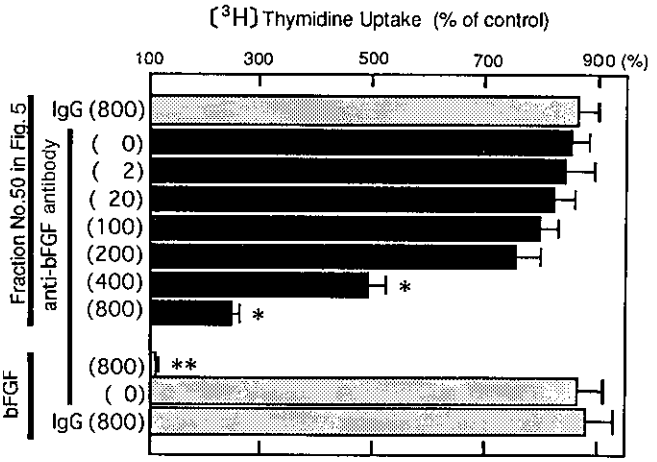

Fig. 8. Inhibitory effect of anti-bFGF antibody on growth-promoting substance from A(−)60 cells. Fraction No. 50 in Fig. 5 (4.3 μg as protein) or bFGF (0.05 ng) was incubated with various amounts of anti-bFGF antibody (2–800 ng) or preimmune rabbit IgG (800 ng) in a total volume of 20 μl. After incubation, the mixture was centrifuged and the supernatant was examined for activity on [^{3}H]thymidine uptake by SC115 cells. Each column represents mean percentage±SE with respect to non-stimulated SC115 cells (3991±190 dpm) from three independent experiments. *, $P<0.01$ against fraction No. 50 without anti-bFGF antibody; **, $P<0.001$ against bFGF without anti-bFGF antibody; IgG, preimmune rabbit IgG.

DISCUSSION

In human prostatic cancer, androgen-insensitive cancer cells grow more rapidly than androgen-sensitive ones.[22] In the present experiments, SC115 cells after long-term removal of testosterone grow rapidly even in serum-free culture without testosterone. Removal of testosterone changed the fibroblastic appearance of SC115 cells to a polygonal form.[23,24] A(−)60 cells also morphologically changed to epithelial-like appearance, and they acquired tumorigenicity. These facts imply that A(−)60 cells had increased phenotypic malignancy, implying progression.

SC115 cells contain approximately three MMTVs in the genome and produce MMTV-related mRNA under stimulation with testosterone.[8] It was reported that after 34 weeks of androgen withdrawal, no MMTV-related mRNA appeared in SC115 cells even after 8 weeks of addition of testosterone, perhaps because of methylation of MMTV-LTR as an androgen responsive element.[8] In the present study, similar disappearance of MMTV-related mRNA occurred, as shown in A(−)60 cells, in spite of addition with testosterone for 12 weeks. There-

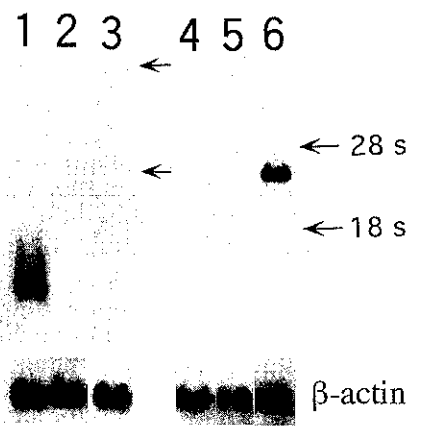

Fig. 9. Northern blot analysis of AIGF and *hst-1*. Poly $(A)^+$ RNAs (2 μg) extracted from SC115 cells (lanes 1, 4), A(−)60 cells (lanes 2, 3, 5), and CS 2 cells (lane 6) cultured with (lanes 1, 3, 4) or without (lanes 2, 5, 6) testosterone were electrophoresed and hybridized with probes of AIGF (lanes 1–3), *hst-1* (lanes 4–6) and β-actin (lower column) using the same filter. Expression of *hst-1* mRNA in CS 2 cells is shown (lane 6). Black arrows, sites of ribosomal RNA.

fore, long-term withdrawal of androgen evokes irreversible changes in SC115 cells.

It was reported that SC115 cells obtained after withdrawal of androgen retained functional androgen receptors even though there was no response to androgen.[25,26] In the present study, long-term removal of testosterone changed SC115 cells to an androgen-insensitive state, accompanied with a slight decrease of androgen binding even though the level of androgen receptor mRNA was maintained. In this context, a previous report from this laboratory showed that CS 2 cells possessed functional androgen receptor with loss of androgen sensitivity.[27] It was reported that the level of estrogen receptor doubled or tripled in estrogen-dependent breast cancer cell lines long after withdrawal of estrogen.[28] The expression level of androgen receptor mRNA was reduced[29] or retained[30] but receptor protein increased in an androgen-sensitive human prostatic cancer cell line, LNCaP cells. Point mutation was detected in androgen receptor gene of LNCaP cells.[31] From these results, the presence of steroid receptor (as judged from either mRNA or receptor protein) does not always imply hormone sensitivity. On the contrary, expression of MMTV-related mRNA disappeared in A(−) cells and this may be a possible explanation of the insensitivity to androgen even in the presence of androgen receptor.

It is generally accepted that the number of FGF receptors correlates with rate of proliferation.[32] Addition of bFGF or testosterone induces an increase in FGF receptor mRNA in SC115 cells.[33] Up-regulation of the receptor mRNA seems to be unusual compared with other receptors, where down-regulation is a common event after binding with ligand.[34] The present study showed an increase of growth-promoting substance(s) secreted from A(−)60 cells, probably bFGF like peptide, and this may cause an increase in number of bFGF receptors. Therefore, increases in growth-promoting substance(s) and in the number of receptors may evoke androgen-insensitive growth of SC115 cells after testosterone withdrawal.

It was recently found that growth of SC115 cells was mediated through AIGF, which is composed of 215 amino acids and shares 30–40% homology with members of the FGF families.[16] CS 2 cells secreted an FGF-like growth factor different from AIGF.[10] In the absence of testosterone, the androgen-independent subline derived from SC115 cells secreted epidermal growth factor-like growth factor, which was sequenced and found to be the mouse homologue of rat schwannoma-derived growth factor (SDGF).[35] In the present study, the growth-promoting substance(s) from A(−)60 cells may be different from SDGF and growth factor secreted from CS 2 cells in terms of elution pattern and inhibition rate with anti-bFGF antibody, respectively. The substance(s) is also different from AIGF, since there was no expression of AIGF mRNA. Thus, the growth-promoting substance(s) from A(−)60 cells may be a new FGF-like factor(s) regulating growth in an autocrine fashion.

In some mouse mammary tumors, integration of MMTV activated *hst-1* and/or *int-2* and promoted tumor formation.[36] Acquired expression of *hst-1* occurred in CS 2 cells and this may be related to androgen-insensitive growth.[21,37] These considerations prompted us to examine *hst-1* and *int-2* mRNA expression, and we found that *hst-1* was not expressed in A(−)60 cells. The level of *int-2* mRNA in A(−)60 cells did not change from that in SC115 cells. Therefore, growth of A(−)60 cells was not mediated by increased expression of oncogenes, which are assumed to be growth-promoting genes, such as *hst-1* and *int-2*.

In conclusion, progression of SC115 cells to androgen-insensitive ones is acquired via production of FGF-like growth factor(s), which might cause an increase in number of bFGF receptors. The sequential step of progression to androgen-insensitive tumor proceeds without androgen, indicating adaptation to androgen-deprived circumstances.

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