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Appearance of Malignant Phenotype with Partial Loss of Hormone Dependency in Androgen-dependent Shionogi Carcinoma 115 Transfected with *hst-1* Gene

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Shionogi Carcinoma 115 (SC 115) and Chiba Subline 2 (CS 2) are an androgen-dependent mouse tumor and an androgen-independent subline derived from SC 115, respectively. Since new expression of the transforming oncogene *hst-1* might be related with autonomous progression in CS 2, the present study was designed to examine the influence of expression of *hst-1* gene on SC 115. The transfectants of SC 115 with *hst-1* still retained androgen sensitivity of growth, although the cells could grow without androgen. The transfectants, however, developed fibroblast-like appearance in the absence of androgen, in contrast to SC 115, which showed epithelial-like appearance after deprivation of androgen. The transfectants acquired an ability to form colonies in soft agar in the absence of androgen. SC 115 could not form tumors in intact mice, but the transfectants formed tumors at a high rate in male mice but not in female ones. From these results, it was concluded that expression of *hst-1* was able to alter the phenotype of the androgen-dependent tumor with partial loss of androgen-dependency, and these changes might be advantageous for malignant progression.

Key words: Shionogi Carcinoma 115 — *hst-1*-transfection — Androgen dependency

Shionogi Carcinoma 115 (SC 115) is a transplantable androgen-dependent tumor of DD/S strain mouse.[1] During serial transplantation of SC 115, an androgen-independent subline, Chiba Subline 2 (CS 2), was obtained.[2-4] SC 115 and CS 2 cells have been cloned with retention of the respective androgen responsiveness and unresponsiveness in our laboratory.[5-7]

The *hst-1* gene was first identified by transfection of NIH/3T3 cells with human DNA samples obtained from gastric cancers.[8,9] Amplification of *hst-1* was reported in esophageal and breast cancers, and it appeared to correlate with clinical stage and prognosis.[10,11] Expression of *hst-1* was also found in some mouse mammary tumors and suggested a relation with tumor growth.[12]

Since it was found that expression of *hst-1* appeared in CS 2 but not in SC 115,[13] *hst-1* was considered to play some role in the progression of androgen-dependent cells to androgen-independent ones. To shed light on the role of *hst-1* in malignant progression, the present study was undertaken to characterize SC 115 cells transfected with *hst-1*.

MATERIALS AND METHODS

Cells Cloning of SC 115 cells was described previously.[5] SC 115 cells were maintained in minimum essential medium(MEM)/Ham's F-12 (1/1, v/v) containing 10% fetal bovine serum (FBS, treated with 0.05% dextran-coated 0.5% charcoal) and 10^{-8} *M* testosterone (maintenance medium). DNA transfection of SC 115 cells was performed by calcium phosphate precipitation with the plasmids pKOneo, pKOc5, and pZB5.[14,15] These plasmids were generous gifts from Dr. M. Terada, National Cancer Center Research Institute. pKOc5 possesses human *hst-1* gene driven by the SV 40 early promoter,[15] and was cotransfected with plasmid DNA containing the bacterial neomycin resistance gene (pKOneo). pZB5 possessed both human *hst-1* gene and neomycin resistance gene driven by murine leukemia virus long terminal repeat.[16] Transfectant with pKOneo was used as a control. After transfection, cells were exposed to medium containing 500 μg/ml of the antibiotic G418 (Gibco, USA), and G418-resistant colonies were isolated, designated as SC 115/neo, SC 115/c5, and SC 115/ZB5, respectively, and used for the study.

To determine cell growth, cells (5×10^4 cells/dishes) were plated onto 35-mm dishes containing 2 ml of maintenance medium. After 48 h, the medium was replaced by MEM/Ham's F-12 (1/1, v/v) containing 1% FBS with or without testosterone, and the medium was changed every other day. Viable cells were counted by trypan blue exclusion using a hemocytometer.

To estimate anchorage-independent growth, 1×10^4 cells in 1 ml of overlayer composed of MEM supplemented with 5% FBS and 0.33% agar were transferred on top of 1 ml of solidified underlayer composed of MEM supplemented with 10% FBS and 0.5% agar in 35-mm dishes. Cells were cultured for three weeks in the presence or absence of 10^{-8} *M* testosterone. Colonies which consisted of more than 50 cells were scored as positive.

DNA and RNA analysis Genomic DNA was obtained by proteinase K treatment followed by phenol extraction, then digested with restriction endonuclease *Hin*dIII.

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Total RNA was prepared by the acid guanidium thiocyanate-phenol-chloroform method.[17] An aliquot of DNA or RNA was electrophoresed on 0.8% or 1% agarose gel, respectively, and transferred onto a nitrocellulose filter by blotting in 20×SSC (1×SSC is 0.15 *M* NaCl and 0.015 *M* sodium citrate). The *Hin*dIII-*Hin*dIII fragment which included the open reading frame of human *hst-1* gene was labeled with ^{32}P-dCTP by means of the multiprime DNA labeling system (Amersham, UK). Southern and Northern blot hybridizations were performed at 42°C for 16 h in 50% formamide, 0.65 *M* NaCl, 0.1 *M* Pipes-NaOH at pH 6.8, 5 m*M* EDTA, 5× Denhardt's solution, 100 μg/ml denatured salmon sperm DNA, 0.1% sodium dodecyl sulfate (SDS), and 10% dextran sulfate.[18] The filters were washed in 0.1×SSC containing 0.1% SDS at 65°C.

Assay for tumorigenicity Cultured cells were harvested with trypsin-EDTA and resuspended in maintenance medium at a density of 2×10^7 cells/ml. Approximately 4×10^6 cells were implanted s.c. at the dorsomedian region of the neck of mice of DD/S strain, 6 to 8 weeks of age. Mice were purchased from Aburabi Farms (Shiga). Four weeks after implantation, the size of the tumors was measured and tumors more than 0.5 cm in diameter were scored as positive.

RESULTS

Expression of *hst-1* in transfectants of SC 115 After culturing SC 115 cells transfected with pKOneo, pKOc5 and pKOneo, or pZB5, respectively, the cells were harvested and the integrated DNA was analyzed. Southern blot analysis of SC 115 cells with an *hst-1* probe showed 3.5 and 1.7 kilobase (kb) fragments, and these fragments were also found in SC 115/c5 and SC 115/ZB5 (Fig. 1). In addition, the fragment of *hst-1* at 0.9 kb in SC 115/c5 and that at 3.0 kb in SC 115/ZB5 were detected, and these had originated from pKOc5 or pZB5, respectively, since they were coincident with the predicted size of *hst-1* derived from pKOc5 or pZB5. Other novel fragments were assumed to be a result of gene rearrangement of the plasmids.

Total RNA samples of the transfectants were analyzed by Northern blot analysis (Fig. 2). The 1.6 and 5.0 kb transcripts were detected in SC 115/c5 and SC 115/ZB5 cells, respectively, but not in SC 115 and SC 115/neo cells. The aberrant sizes of the *hst-1* transcripts could be due to precursor RNA or products formed by alternative splicing. The same blot was reprobed for β-actin mRNA to confirm that similar amounts of RNA had been loaded.

Cell growth SC 115 and SC 115/neo cells grew exponentially in the presence of 10^{-8} *M* testosterone, and the growth rate of SC 115/neo was almost identical to

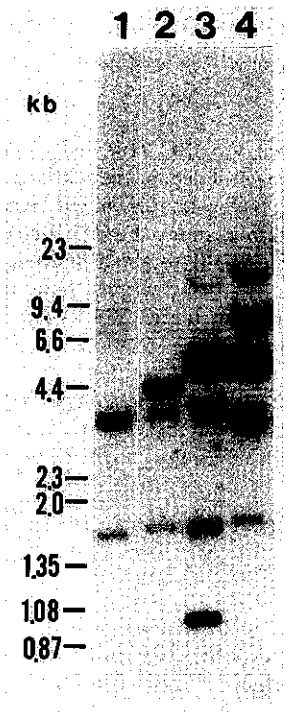

Fig. 1. Southern blot analysis of genomic DNA from SC 115 and transfectants of *hst-1* and/or neomycin resistance genes. DNA samples (10 μg/lane) from SC 115 (lane 1), SC 115/neo (lane 2), SC 115/c5 (lane 3), and SC 115/ZB5 (lane 4) were digested with *Hin*dIII and analyzed with a probe specific for the open reading frame of the human *hst-1* gene. *Hin*dIII digest of λDNA and *Hae*III digest of φX174 were used as markers.

that of SC 115 (Fig. 3). In the absence of testosterone, they did not proliferate, showing androgen-dependent growth. Growth rates of SC 115/c5 and SC 115/ZB5 in the presence of testosterone were significantly higher than those of SC 115 and SC 115/neo cells. These two cell lines showed growth even in the absence of testosterone, but addition of testosterone evoked more rapid proliferation, indicating a response to androgen in these cells.

Colony formation in soft agar was examined. SC 115 and SC 115/neo cells did not proliferate without testosterone. On the contrary, approximately 10 colonies/dish appeared in cultures of SC 115 and SC 115/neo in the presence of testosterone (Table I). SC 115/c5 and SC 115/ZB5 formed colonies in the absence of testosterone, but the effect of testosterone on colony formation was evident. These results showed that changes of androgen dependency of growth occurred after transfection of SC 115 cells with *hst-1*.

Morphology of transfectants SC 115 cells showed fibroblast-like shape in the presence of androgen but

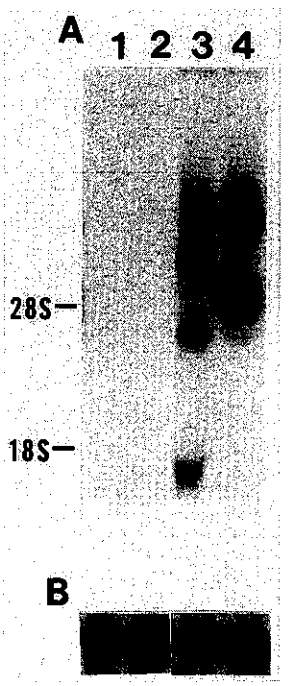

Fig. 2. Northern blot analysis of RNA from SC 115 and transfectants of *hst-1* and/or neomycin resistance genes. Total RNAs (20 μg/lane) from SC 115 (lane 1), SC 115/neo (lane 2), SC 115/c5 (lane 3), and SC 115/ZB5 (Lane 4) were applied. Hybridization was performed with probes of *hst-1* (A) and β-actin (B). Positions of ribosomal RNA are indicated.

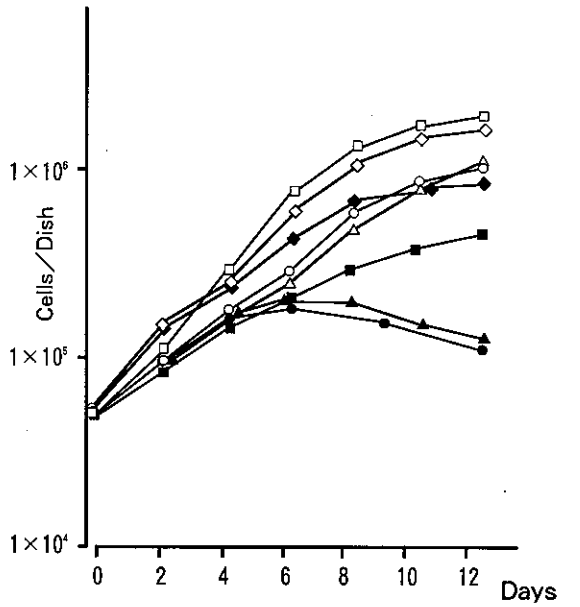

Fig. 3. Growth of SC 115 cells and transfectants of *hst-1* and/or neomycin resistance genes. Cells were cultured in maintenance medium for 2 days and transferred to MEM/Ham's F-12 containing 1% FBS with (open symbols) or without (closed symbols) 10^{-8} *M* testosterone: SC 115 (triangles), SC 115/neo (circles), SC 115/c5 (diamonds) and SC 115/ZB5 (squares). Each point was calculated from three dishes; SE is not depicted due to its very low level.

removal of testosterone changed the shape to epithelial-like morphology as evaluated by phase contrast microscopy (Fig. 4). The shape of SC 115/neo and the influence of testosterone on the cells were almost identical to those of SC 115 cells. SC 115/c5 and SC 115/ZB5 showed a fibroblast-like appearance regardless of the presence or absence of testosterone. The latter two formed distinct foci in which the cells were piled up.

Tumorigenicity in mice After transplantation of SC 115 and SC 115/neo cells, small nodules less than 0.5 cm in diameter appeared in a few animals, but further growth of nodules did not occur during 12 weeks, and they were assumed to be non-tumorous (Table II). On the contrary, SC 115/c5 and SC 115/ZB5 formed tumors in male mice. The tumors which arose after inoculation of SC 115/c5 cells were poorly differentiated adenocarcinomas (Fig. 5). The histological appearance of SC 115/ZB5 was similar to that of SC 115/c5 (data not shown). SC 115/c5 and SC 115/ZB5 scarcely formed tumors in female mice, showing dependency upon androgen. Metastatic foci did not appear in mice bearing tumors of SC 115/c5 and SC 115/ZB5 up to 12 weeks during the experimental period.

Table I. Anchorage-independent Growth of SC 115 Cells and Transfectants of *hst-1* and/or Neomycin Resistance Genes

Cell line	Colonies/dish	
	Testosterone[a]	
	(−)	(+)
SC 115	0	8±2
SC 115/neo	0	6±1
SC 115/c5	24±5	174±15
SC 115/ZB5	28±5	60±10

a) The cells were cultured in soft agar with or without 10^{-8} *M* testosterone.

DISCUSSION

It was reported that *hst-1* was expressed in some mouse mammary tumors of BR6 strain with integrated mouse mammary tumor virus (MMTV).[12] Integration of MMTV was found in both SC 115 and CS 2 in identical sites, and only the latter expressed *hst-1* without new insertion of MMTV.[13]

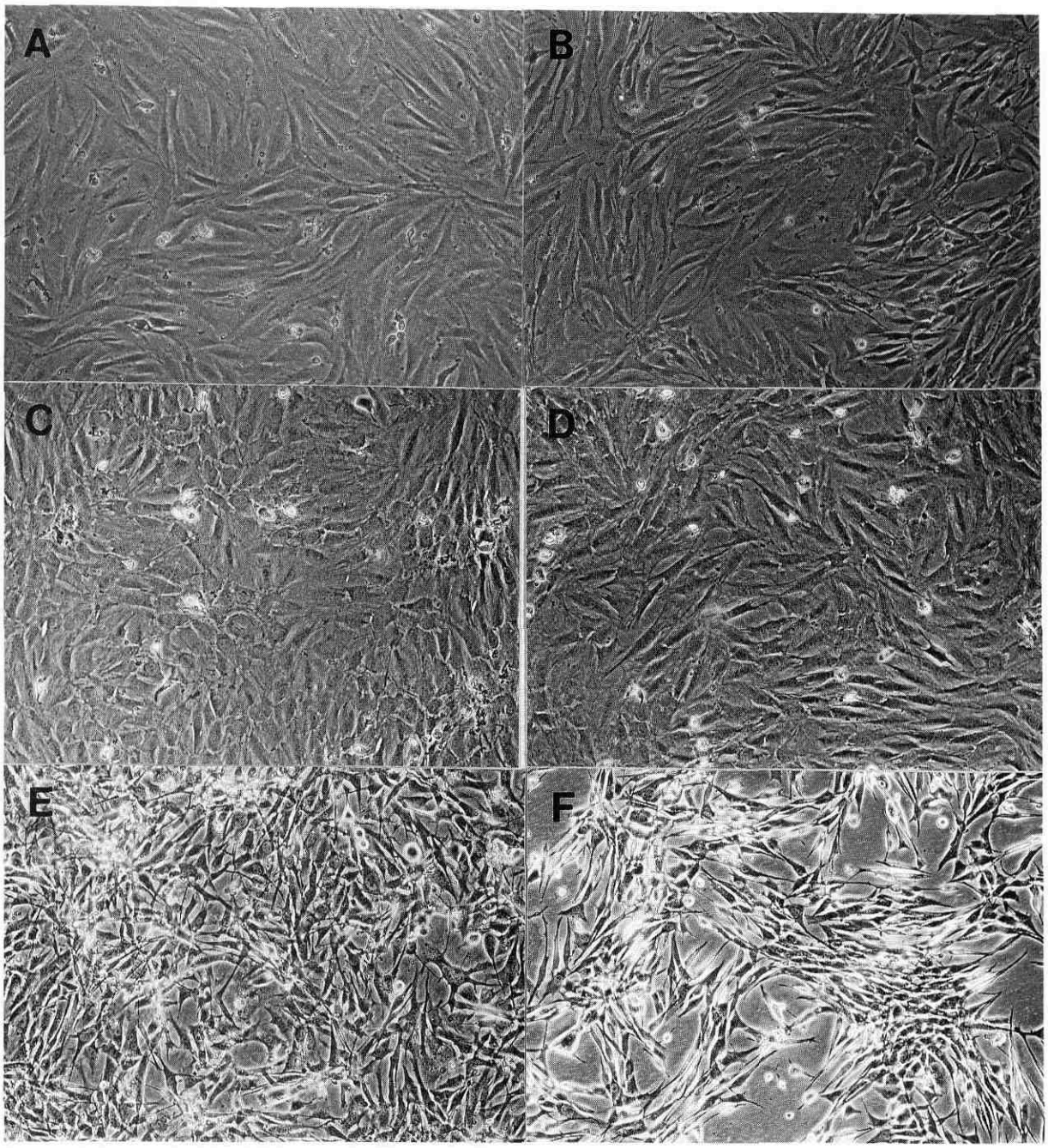

Fig. 4. Morphology of SC 115 cells and transfectants of *hst-1* and/or neomycin resistance genes. SC 115 (A, B), SC 115/neo (C, D), SC 115/c5 (E), and SC 115/ZB5 (F) were cultured in maintenance medium for 2 days and transferred to MEM/Ham's F-12 containing 1% FBS in the absence (A, C, E, F) or presence (B,D) of testosterone. After 4 days, the cells were photographed. ×100.

SC 115/c5 and SC 115/ZB5, transfectants with *hst-1*, acquired proliferating ability in anchorage-dependent and -independent growth without androgen. Although the transfectants preserved androgen-responsiveness of growth, they might have malignant potential when compared with the parent SC 115 cells. It was reported that

Table II. Tumorigenicity of SC 115 Cells and Transfectants of *hst-1* and/or Neomycin Resistance Genes

Cell line	Mice with tumors[a]			
	male		female	
SC 115	0/20	(0)[b]	0/20	(0)
SC 115/neo	0/11	(0)	0/9	(0)
SC 115/c5	10/10	(100)	0/8	(0)
SC 115/ZB5	8/10	(80)	1/9	(11)

a) Mice given s.c. injections of 4×10^6 cells.
b) % tumor formation.

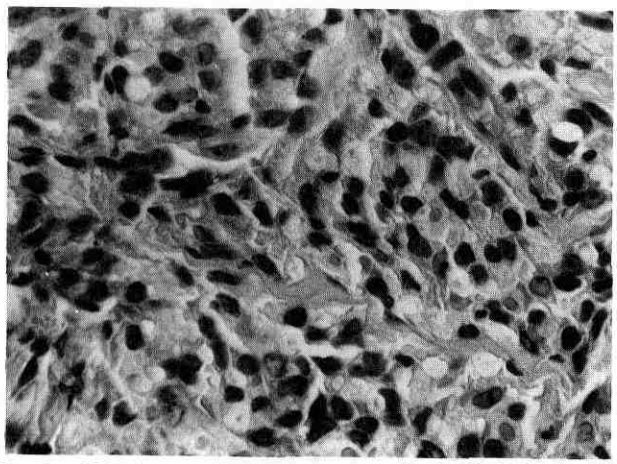

Fig. 5. Histology of tumors composed of SC 115/c5 cells. Approximately 4×10^6 cells were inoculated into DD/S mice and 4 weeks after transplantation, tumors were excised. H & E, ×400.

SC 115 cells could not grow in intact male mice without a pharmacological dose of exogenous testosterone.[19] The present study also confirmed no growth of SC 115 in intact mice. Only one tumor (11%) appeared in a female mouse inoculated with SC 115/ZB5. This might be explained by the assumption that an androgen-insensitive mutant appeared during transplantation. Acquired malignant potential in the transfectants seems to be related with expression of *hst-1*, since control transfectants with pKOneo did not show different characteristics from SC 115 cells.

Recently it was reported that in serum-free culture, the growth of SC 115 cells was regulated by an androgen-induced fibroblast growth factor (FGF)-like peptide in an autocrine fashion.[20-22] CS 2 cells also secreted a similar FGF-like peptide which promoted the growth of themselves and of the parent SC 115 cells in autocrine and paracrine manners, respectively.[6,7] The FGF-like peptides secreted from both SC 115 and CS 2 cells may share the same receptors with FGFs,[7,22] but the peptides differed from authentic FGFs in their physicochemical properties. Moreover, the peptide from CS 2 cells was not identical in molecular weight with that secreted from SC 115.[7,22] From the DNA sequence of *hst-1* in human cancers, it was predicted that the product of *hst-1* consisted of 206 amino acids which had about 40% homology to FGFs.[15,23] The murine homologue of the *hst-1* gene was sequenced, and it encoded a protein which consisted of 202 amino acids and had 82% homology to human *hst-1*.[24] The transfectants produced growth-promoting factor, since conditioned media obtained from SC 115/c5 and SC 115/ZB5 in the absence of androgen had marked growth-promoting activity toward SC 115 cells (data not shown). The molecular weight of FGF-like peptide from CS 2 was assumed to be approximately 50,000,[6] and therefore FGF-like peptide seems to be different from the product of *hst-1*, although CS 2 expressed *hst-1*. The nature of FGF-like peptides secreted by SC 115 and CS 2 has not been clarified yet, but it is probable that these peptides belong to the FGF family.

FGFs are potent mitogens for a variety of cells and also play important roles in tumor development and angiogenetic processes.[25-27] A different growth property of CS 2 was noticed with a less necrotic tendency when compared with SC 115,[5] and FGF-like peptide produced by CS 2 may contribute to the prevention of the necrotic process. Expression of *hst-1* seems to offer some advantages over SC 115 for tumor growth in mice.

There have been reports indicating that expression of some oncogenes alters the hormone sensitivity of tumors. When MCF-7 cells, an estrogen-dependent human mammary carcinoma cell line, were transfected with v-H-*ras*, the transfectants showed estrogen-independent growth and an ability to form tumors in nude mice in the absence of estrogen.[28-30] It was reported that expression of H-*ras* oncogene overcame hormone-dependent growth of MCF-7 cells *in vitro*, but not *in vivo*.[31] On the contrary, Sommers *et al.*[32] reported that H-*ras*-transfected MCF-7 cell lines were estrogen-responsive for cell growth in culture but some cell lines formed tumors in the absence of estradiol in nude mice. Therefore, some discrepancies concerning autonomy exist in the case of MCF-7 cells transfected with *ras* oncogene, but it should be stressed that alteration of hormone sensitivity is evoked by new expression of some oncogenes in hormone-dependent tumors.

From the present study, we conclude that new expression of *hst-1* gene in SC 115 assists malignant progression, but the transfectants do not achieve complete escape from response to androgen. This may imply that loss of growth dependency on androgen is not a single-step change.

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REFERENCES

1) Minesita, T. and Yamaguchi, K. An androgen-dependent tumor derived from a hormone-independent spontaneous tumor of a female mouse. *Steroids*, **4**, 815–830 (1964).
2) Fuse, H., Akimoto, S., Sato, R., Miyauchi, T., Wakisaka, M., Hosoya, T. and Shimazaki, J. Changes in cytosolic androgen receptor after administration of testosterone of androgen-dependent mouse mammary tumor (Shionogi Carcinoma) and its sublines of altered androgen dependency. *Endocrinol. Jpn.*, **30**, 189–197 (1983).
3) Suzuki, N., Urata, M., Miyauchi, T., Wakisaka, M., Shimazaki, J. and Hosoya, T. *In vivo* effect of androgen on RNA synthesis in nuclei from androgen-independent subline of Shionogi Carcinoma (CS 2). *Endocrinol. Jpn.*, **30**, 657–661 (1983).
4) Zama, S., Akimoto, S., Yazawa, S., Ichikawa, T., Hayata, I., Petrow, V. and Shimazaki, J. Androgen receptor, testosterone uptake and karyotype in androgen-dependent mouse tumor (SC 115) and its androgen-independent subline (CS 2). *Endocrinol. Jpn.*, **34**, 279–289 (1987).
5) Ichikawa, T., Akimoto, S., Hayata, I. and Shimazaki, J. Progression and selection in heterogeneous tumor composed of androgen-responsive Shionogi Carcinoma 115 and its autonomous subline (Chiba Subline 2). *Cancer Res.*, **49**, 367–371 (1989).
6) Furuya, Y., Sato, N., Akakura, K., Ichikawa, T., Suzuki, N., Sato, R. and Shimazaki, J. Paracrine growth stimulation of androgen-responsive Shionogi Carcinoma 115 by its autonomous subline (Chiba Subline 2). *Cancer Res.*, **50**, 4979–4983 (1990).
7) Furuya, Y., Sato, N., Watabe, Y. and Shimazaki, J. Effect of suramin on growth of androgen-responsive mouse tumor (Shionogi Carcinoma 115) and its autonomous subline (Chiba Subline 2). *Endocrinol. Jpn.*, **37**, 933–942 (1990).
8) Sakamoto, H., Mori, M., Taira, M., Yoshida, T., Matsukawa, S., Shimizu, K., Sekiguchi, M., Terada, M. and Sugimura, T. Transforming gene from human stomach cancers and a noncancerous portion of stomach mucosa. *Proc. Natl. Acad. Sci. USA*, **83**, 3997–4001 (1986).
9) Sakamoto, H., Yoshida, T., Nakakuki, M., Odagiri, H., Miyagawa, K., Sugimura, T. and Terada, M. Cloned *hst* gene from normal human leukocyte DNA transforms NIH3T3 cells. *Biochem. Biophys. Res. Commun.*, **151**, 965–972 (1988).
10) Tsuda, T., Tahara, E., Kajiyama, G., Sakamoto, H., Terada, M. and Sugimura, T. High incidence of coamplification of *hst-1* and *int-2* genes in human esophageal carcinomas. *Cancer Res.*, **49**, 5505–5508 (1989).
11) Tsuda, H., Hirohashi, S., Shimosato, Y., Hirota, T., Tsugane, S., Yamamoto, H., Miyajima, N., Toyoshima, K., Yamamoto, T., Yokota, J., Yoshida, T., Sakamoto, H., Terada, M. and Sugimura, T. Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene-coamplification units: *hst-1/int-2* and c-*erb*B-2/*ear*-1. *Cancer Res.*, **49**, 3104–3108 (1989).
12) Peters, G., Brookes, S., Smith, R., Placzek, M. and Dickson, C. The mouse homolog of the *hst/k-FGF* gene is adjacent to *int-2* and is activated by proviral insertion in some virally induced mammary tumors. *Proc. Natl. Acad. Sci. USA*, **86**, 5678–5682 (1989).
13) Akakura, K., Furuya, Y., Sato, N., Kodama, T., Teshima, S., Shimosato, Y., Yoshida, T., Terada, M. and Shimazaki, J. Acquired expression of *hst-1* in an autonomous subline (Chiba Subline 2) derived from androgen-responsive mouse mammary tumor (Shionogi Carcinoma 115). *Jpn. J. Cancer Res.*, **81**, 554–556 (1990).
14) Parker, B. A. and Stark, G. R. Regulation of simian virus 40 transcription: sensitive analysis of the RNA species present early in infections by virus or viral DNA. *J. Virol.*, **31**, 360–369 (1979).
15) Taira, M., Yoshida, T., Miyagawa, K., Sakamoto, H., Terada, M. and Sugimura, T. cDNA sequence of human transforming gene *hst* and identification of the coding sequence required for transforming activity. *Proc. Natl. Acad. Sci. USA*, **84**, 2980–2984 (1987).
16) Cepko, C. L., Roberts, B. E. and Mulligan, R. C. Construction and applications of a highly transmissible murine retrovirus shuttle vector. *Cell*, **37**, 1053–1062 (1984).
17) Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159 (1987).
18) Sambrook, J., Fritsch, E. F. and Maniatis, T. "Molecular Cloning," 2nd Ed., pp. 6.3–7.52 (1989). Cold Spring Harbor Lab. Press, Cold Spring Harbor.
19) Terada, N., Yamamoto, R., Uchida, N., Takada, T., Taniguchi, H., Takatsuka, D., Sawada, M., Tsuji, M., Li, W., Kitamura, Y. and Matsumoto, K. Androgen dependency of a tumor produced by a cell line derived from androgen-responsive Shionogi Carcinoma 115. *Cancer Res.*, **49**, 693–698 (1989).
20) Nonomura, N., Nakamura, N., Uchida, N., Noguchi, S., Sato, B., Sonoda, T. and Matsumoto, K. Growth-stimulatory effect of androgen-induced autocrine growth

factor(s) secreted from Shionogi Carcinoma 115 cells on androgen-unresponsive cancer cells in a paracrine mechanism. *Cancer Res.*, **48,** 4904–4908 (1988).
21) Lu, J., Nishizawa, Y., Tanaka, A., Nonomura, N., Yamanishi, H., Uchida, N., Sato, B. and Matsumoto, K. Inhibitory effect of antibody against basic fibroblast growth factor on androgen- or glucocorticoid-induced growth of Shionogi Carcinoma 115 cells in serum-free culture. *Cancer Res.*, **49,** 4963–4967 (1989).
22) Nonomura, N., Lu, J., Tanaka, A., Yamanishi, H., Sato, B., Sonoda, T. and Matsumoto, K. Interaction of androgen-induced autocrine heparin-binding growth factor with fibroblast growth factor receptor on androgen-dependent Shionogi Carcinoma 115 cells. *Cancer Res.*, **50,** 2316–2321 (1990).
23) Yoshida, T., Miyagawa, K., Odagiri, H., Sakamoto, H., Little, P. F. R., Terada, M. and Sugimura, T. Genomic sequence of *hst*, a transforming gene encoding a protein homologous to fibroblast growth factors and the *int-2*-encoded protein. *Proc. Natl. Acad. Sci. USA*, **84,** 7305–7309 (1987).
24) Brookes, S., Smith, R., Thurlow, J., Dickson, C. and Peters, G. The mouse homologue of *hst/k*-FGF: sequence, genome organization and location relative to *int-2*. *Nucleic Acids Res.*, **17,** 4037–4045 (1989).
25) Gospodarowicz, D., Neufeld, G. and Schweigerer, L. Fibroblast growth factor. *Mol. Cell. Endocrinol.*, **46,** 187–204 (1986).
26) Lobb, R. R. and Fett, J. W. Purification of two distinct growth factors from bovine neural tissue by heparin affinity chromatography. *Biochemistry*, **23,** 6295–6299 (1984).
27) Gospodarowicz, D., Baird, A., Cheng, J., Lui, G. M., Esch, F. and Bohlen, P. Isolation of fibroblast growth factor from bovine adrenal gland: physicochemical and biological characterization. *Endocrinology*, **118,** 82–90 (1986).
28) Kasid, A., Lippman, M. E., Papageorge, A. G., Lowy, D. R. and Gelmann, E. P. Transfection of v-ras^H DNA into MCF-7 human breast cancer cells bypasses dependence on estrogen for tumorigenicity. *Science*, **228,** 725–728 (1985).
29) Kasid, A., Knabbe, C. and Lippman, M. E. Effect of v-ras^H oncogene transfection on estrogen-independent tumorigenicity of estrogen-dependent human breast cancer cells. *Cancer Res.*, **47,** 5733–5738 (1987).
30) Dickson, R. B., Kasid, A., Huff, K. K., Bates, S. E., Knabbe, C., Bronzert, D., Gelmann, E. P. and Lippman, M. E. Activation of growth factor secretion in tumorigenic states of breast cancer induced by 17β-estradiol or v-Ha-*ras* oncogene. *Proc. Natl. Acad. Sci. USA*, **84,** 837–841 (1987).
31) Sukumar, S., Carney, W. P. and Barbacid, M. Independent molecular pathways in initiation and loss of hormone responsiveness of breast carcinomas. *Science*, **240,** 524–526 (1988).
32) Sommers, C. L., Papageorge, A., Wilding, G. and Gelmann, E. P. Growth properties and tumorigenesis of MCF-7 cells transfected with isogenic mutants of ras^H. *Cancer Res.*, **50,** 67–71 (1990).