Acquired Expression of *hst-1* in an Autonomous Subline (Chiba Subline 2) Derived from Androgen-responsive Mouse Mammary Tumor (Shionogi Carcinoma 115)

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Since growth of Shionogi Carcinoma 115 (SC 115) and its autonomous subline (CS 2) were regulated by fibroblast growth factor-like peptide, expression of *int-2* and *hst-1* was examined in these cell lines. Hybridization of genomic DNA with long terminal repeat of mouse mammary tumor virus (MMTV) revealed the same pattern of restriction fragments, showing the same integration of MMTV. Although weak expression of *int-2* was noticed in the two cells, clear expression of *hst-1* was seen only in CS 2 cultured with/without testosterone. It is suggested that autonomous growth of androgen-unresponsive CS 2 is connected with expression of *hst-1*.

Key words: Shionogi Carcinoma 115 — Chiba Subline 2 — *int-2* — *hst-1*

Shionogi Carcinoma 115 (SC 115) has been accepted as an androgen-responsive mouse mammary tumor.^{1,2)} Recently it was reported that in serum-free culture, growth of SC 115 was regulated by androgen-induced fibroblast growth factor (FGF)-like peptide in an autocrine manner.^{3,4)} Chiba Subline 2, derived from SC 115, was an androgen-unresponsive autonomous subline,^{5,6)} and secreted a similar FGF-like growth factor which promoted the growth of CS 2 and parent SC 115 in autocrine and paracrine manners, respectively.⁷⁾ Although SC 115 arose in a mouse of DDS strain, insertion of proviral DNA of mouse mammary tumor virus (MMTV) was found in genomic DNA of SC 115.⁸⁾ A recent report indicated that *int-2* and/or *hst-1* gene was expressed in some mouse mammary tumors induced by integration of MMTV.⁹⁾ It has been claimed that *int-2* and *hst-1* genes belong to the FGF gene family.^{10,11)} Since, among various growth factors including EGF, insulin, PDGF and TGF- β , FGF alone showed growth-stimulating activity towards cloned cells from SC 115,¹²⁾ we became interested in the role of oncogenes producing FGF-like substances. Thus, the present study was undertaken to examine the expression of mouse *int-2* and *hst-1* genes in SC 115 and CS 2.

Cells (5×10^5) of the cloned cell lines from SC 115 or CS 2 were plated on a 100 mm dish and cultured in MEM:Ham's F-12 (1:1) medium supplemented with 10% fetal bovine serum (treated with dextran-coated charcoal) for 2 days, then the medium was changed to

the medium without fetal bovine serum (serum-free medium). When necessary, 10^{-8} M testosterone was added to the medium. Genomic DNA was extracted by proteinase K treatment followed by phenol extraction, then digested with restriction enzyme, *EcoRI* or *MspI*. Total RNA was prepared by guanidium thiocyanate extraction and centrifugation in cesium chloride, then poly(A)⁺RNA was separated using Oligotex-dT30 (Takara, Kyoto). An aliquot of DNA or poly(A)⁺RNA was electrophoresed on 0.8% or 1.0% agarose gel, respectively, and transferred onto a nitrocellulose filter by blotting in $20 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The following probes were used: a 1.4 kilobase (kb) *PstI*-*PstI* fragment from long terminal repeat (LTR) of MMTV for Southern blotting,⁸⁾ a 0.6 kb *SacI*-*EcoRI* fragment (*int-2* f) from the third exon of mouse *int-2* gene,¹³⁾ and a 1.8 kb *EcoRI*-*EcoRI* fragment (M 1.8) which included the third exon of mouse *hst-1* gene.¹⁴⁾ The probes were labeled with ³²P-dCTP by means of the multiprime DNA labeling system for MMTV-LTR (Amersham, UK) or by nick translation for *int-2* f and M 1.8 (Takara). Southern and Northern blot hybridizations were performed by standard methods¹⁵⁾ and the filter was washed in 0.2 (Southern) or 0.1 (Northern) $\times$ SSC containing 0.1% sodium dodecyl sulfate at 65°C.

Growth of CS 2 was not influenced by addition of testosterone. As SC 115 is androgen-responsive, culturing in serum-free medium required testosterone. The growth patterns of SC 115 and CS 2 were almost identical and exponential proliferation was seen up to the 8 th day after the start of culture.¹⁶⁾ Cells were harvested at the 6th day for analysis of DNA and RNA.

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Fig. 1. Southern blot analysis of genomic DNA from SC 115 (A) and CS 2 (B). Genomic DNA was extracted, digested with *Eco*RI (lane 1) or *Msp*I (lane 2) or not digested (lane 3), and 7.3 μ g of DNA/lane was electrophoresed. Hybridization was performed with a probe of MMTV-LTR. *Hind*III digest of λ DNA and *Hae*III digest of ϕ X174 were used as markers.

Restriction fragments hybridized with MMTV-LTR were almost identical between SC 115 and CS 2 after digestion with *Eco*RI or *Msp*I (Fig. 1). The same lengths of hybridized fragments with MMTV-LTR were reported in SC 115,⁸⁾ so insertion of MMTV in SC 115 seems to be stable. Moreover, no new insertion of MMTV-LTR sequence was found in genomic DNA of CS 2 as compared with that of SC 115.

Although weak expression of *int-2* gene was observed in both SC 115 and CS 2 by Northern blot hybridization, the level of expression seemed to be less in SC 115 than in CS 2 (Fig. 2A). In CS 2, expression of *int-2* was not influenced by addition of testosterone. It is known that four different sizes of messenger RNA corresponding to *int-2* gene, approximately 2.9, 2.6, 1.7 and 1.4 kb, are present in mouse endodermal cells,¹⁷⁾ while in the present experiment one hybridized band with *int-2* f probe was found at 2.7 kb. Other bands of different sizes were not obtained, and this may be attributable to the low level of expression of *int-2*.¹⁸⁾

Fig. 2. Northern blot analysis of poly(A)⁺RNA obtained after culturing SC 115 (lane 1) or CS 2 in the presence (lane 2) or absence (lane 3) of 10^{-8} M testosterone. For electrophoresis, 3.0 μ g of poly(A)⁺ RNA/lane was applied. Hybridization was performed with probes of *int-2* (A), *hst-1* (B) and actin (C) using the same filter. Arrows indicate positions of transcripts of *int-2* (2.7 kb) and *hst-1* (3.2 kb). Positions of ribosomal RNA (28S, 18S) are indicated.

The 3.2 kb transcript of mouse *hst-1* gene was clearly expressed in CS 2 (Fig. 2B), but there was no detectable hybridized band with *hst-1* in SC 115. Addition of testosterone did not influence the expression of *hst-1* in CS 2. Throughout Northern blot analyses, approximately equal amounts of expression of actin-messenger RNA were seen (Fig. 2C).

The MMTV-LTR contains hormone-responsive elements for androgen and glucocorticoid,¹⁹⁾ and it was reported that androgen accumulated LTR-related RNA in SC 115 via androgen receptor.²⁰⁾ Many mammary tumors in BR6 strain of mice contain MMTV insertion and consequently a part of them expressed *int-2* gene.²¹⁾ Weak expression of *int-2* in SC 115 shown in the present report may correlate with growth regulation induced by androgen through MMTV-LTR. Although weak expression of *int-2* gene was present, CS 2 showed remarkable transcription of *hst-1*. The product of *hst-1* shows growth-promoting activity in NIH 3T3 cell line.²²⁾ Therefore, expression of *hst-1* seems to be related to the autonomous growth of CS 2 uninfluenced by androgen. It was reported that mouse *hst-1* gene was mapped adjacent to *int-2* gene on chromosome 7.⁹⁾ Although SC 115 and CS 2 share several common marker chromosomes, the modal number in CS 2 was 72 with trisomy of no. 7.²³⁾ Thus, aberrance of chromosome 7 might cause expression of *hst-1*. Transcription of both *int-2* and *hst-1* was rare, but was observed in a few mouse mammary tumors.⁹⁾ The present data on CS 2 also include a case of coexpression.

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