



Transforming Growth Factor- α mRNA and Epidermal Growth Factor Receptor mRNA Expression in Normal and Neoplastic Mammary Glands of Four Strains of Mice with Different Mammary Tumor Potentials

Authors: Tsunoda, Satoshi, Ikezaki, Yoshie, and Nagasawa, Hiroshi

Source: Zoological Science, 14(5) : 803-807

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.14.803>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Transforming Growth Factor- α mRNA and Epidermal Growth Factor Receptor mRNA Expression in Normal and Neoplastic Mammary Glands of Four Strains of Mice with Different Mammary Tumor Potentials

Satoshi Tsunoda, Yoshie Ikezaki and Hiroshi Nagasawa*

Experimental Animal Research Laboratory, Meiji University, Tama-ku, Kawasaki 214-71, Japan

ABSTRACT—Transforming growth factor- α (TGF α) and epidermal growth factor receptor (EGF-R) mRNAs were determined by reverse transcriptase-polymerase chain reaction (RT-PCR) in the normal and neoplastic mammary glands of four strains of mice with different mammary tumor potentials (from highest to lowest potential): SHN, GR/A, SLN and C3H/He. At 2 months of age, when the mammary glands of these strains consisted mostly of normal tissue, the samples examined showed the positive expressions of both TGF α and EGF-R mRNAs in all strains (4-6 mice per group), except for EGF-R mRNA in the SLN mice, expressed in only 2 of 4 samples associated with no end-bud formation in the mammary glands. At 10 months, all of the samples from all four strains had a positive expression of TGF α mRNA. The EGF-R mRNA expression paralleled the degree of the formation of preneoplastic hyperplastic alveolar nodules (HAN) in all strains. These findings indicate that TGF α and EGF-R participate in the growth of the mammary glands, and that EGF-R especially contributes to the formation of end-buds at younger ages and to that of preneoplastic HAN at later ages. All of the samples of mammary tumors from four strains had positive expressions of both TGF α and EGF-R mRNAs.

INTRODUCTION

Transforming growth factor- α (TGF α) is a 50 amino acid polypeptide (Derynck *et al.*, 1984; Lee *et al.*, 1985) that shares approximately 30% homology with epidermal growth factor (EGF) and acts through binding to EGF receptor (EGF-R) (Todaro *et al.*, 1980; Derynck, 1988). TGF α has been identified in normal mammary glands and mammary tumors *in vivo* and *in vitro* (Pimentel, 1994a; Dickson and Lippman, 1995; Ethier, 1995). Furthermore, Matsui *et al.* (1990), Halter *et al.* (1992) and Mizuno *et al.* (1994) reported the stimulated growth of normal, preneoplastic and neoplastic mammary glands in human TGF α transgenic mice.

EGF-R is a 1,163 amino acid polypeptide, localized on the surface of cells including mammary epithelial cells and mammary tumor cells. EGF-R has tyrosine kinase activity, and closely resembles the oncogene v-erb-B (Pimentel, 1994b). The overexpression of EGF-R in human breast cancer is related to the loss of hormone dependency and poor prognosis (Pimentel, 1994b; Dickson and Lippman, 1995).

Taken together, the above findings indicate the significant participation of TGF α and EGF-R in the normal and neoplastic growth of mammary glands (Kenney and Dickson,

1996).

Four strains of mice maintained in our laboratory are characteristic in having different potentials for normal and neoplastic mammary gland growth higher in order of SHN, GR/A, SLN and C3H/He (Nagasawa *et al.*, 1987a, b).

In this study, the TGF α mRNA and EGF-R mRNA expressions in normal mammary glands and mammary tumors were compared among these four strains of mice.

MATERIALS AND METHODS

Animals

The SHN/Mei, SLN/Mei (Nagasawa *et al.*, 1976; Staats, 1985) GR/AMei (Mühlbock, 1965; Yanai and Nagasawa, 1978) and C3H/HeMei (Nagasawa *et al.*, 1979) strains of mice were used. They were maintained by strict brother \times sister mating, kept in aluminum cages (18 \times 30 \times 15 cm) with wood shavings (M size: CLEA JAPAN, Tokyo, Japan), 4-6 each, maintained in a windowless animal room, which was air-conditioned (21-22°C and 65-70% relative humidity) and artificially illuminated (14 hr of light from 05:00 to 19:00) and ventilated (16 times/hr). The mice were provided with a commercial diet (Lab MR Breeder: Nihon Nosan Kogyo KK, Yokohama, Japan) and tap water *ad libitum*.

In each strain, virgin mice were killed at 2 and 10 months of age by decapitation under light ether anesthesia. The retired mice checked for palpable mammary tumors were also killed in a week after the first tumor appearance (tumor sizes were 3-5 mm).

All procedures were carried out according to the NIH Guide for the Care and Use of Laboratory Animals, USA.

* Corresponding author: Tel. +81-44-934-7073;
FAX. +81-44-934-7073.

Normal and preneoplastic mammary gland growth

The unilateral third thoracic gland was fixed in Bouin's solution, prepared for wholemount evaluation and examined under 10-fold magnification. The degree of the formation of normal end-buds was rated from 1 to 7 in increments of 1 (Nagasawa *et al.*, 1980) and the area bound the tops of ducts by the straight lines was also measured automatically by a computerized digitizer (Model LA-525; PLAS, Tokyo, Japan) as an index of duct extension. The number of preneoplastic mammary hyperplastic alveolar nodules (HAN) was counted, and the area of each HAN was also measured by the digitizer.

RNA isolation

Bilateral inguinal mammary glands and a portion of each mammary tumor with the least necrosis were frozen immediately after killing and kept at -70°C . The total RNA in each sample was extracted by the acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987).

Detection of TGF α mRNA and EGF-R mRNA expressions

The TGF α mRNA and EGF-R mRNA expressions were detected by the reverse transcriptase-polymerase chain reaction (RT-PCR) method. Reverse-transcribed cDNA was synthesized with the use of a cDNA synthesis kit (TaKaRa, Kyoto, Japan) with RAV-2 reverse transcriptase and random primers, and the target cDNA was amplified by PCR with a PCR Amplification kit (TaKaRa) with *Taq* DNA polymerase.

The sense and antisense primers derived from the mouse TGF α cDNA sequence (Vaughan *et al.*, 1992) were used for a PCR reaction for the detection of TGF α mRNA expression. The conditions were the same as detailed previously (Harigaya *et al.*, 1994a).

The PCR reaction for the detection of EGF-R mRNA expression was performed for 40 cycles (1 cycle = 94°C for 1 min, 55°C for 1 min, 72°C for 2 min). The sense and antisense primers derived from the mouse EGF-R cDNA sequence (Avivi *et al.*, 1991; Luetkeke *et al.*, 1994) were at nucleotides 236-255 (5'-GGAGGAAAAGAAAGTCT-GCC-3') and 539-520 (5'-CCCATAGTTGGATAGATGG-3'), respectively (Paria *et al.*, 1993). Other conditions were the same as those for the detection of TGF α mRNA expression.

β -Actin primers derived from the mouse sequence (Alonso *et al.*, 1986) were used for the RT-PCR control reaction as reported by Harigaya *et al.* (1994a).

Statistics

The significance of differences in the frequency of TGF α mRNA and EGF-R mRNA expression among the strains was evaluated by χ^2 -test. For other parameters, Duncan's Multiple range test was used.

RESULTS

Normal and preneoplastic mammary gland growth (Table 1)

At 2 months of age, mammary rating of SLN was 1-2 indicating that the glands consisted mostly of ducts with no end-buds and it was significantly lower than those of the other three strains (2-4). A significant difference was not seen among the strains in the mammary gland area. The formation of HAN was not detected in any strain.

At 10 months of age, the rating was significantly lower in the SLN and GR/A mice than in the SHN mice. Mammary gland area differed little among the strains.

The number of HAN was the highest in the SHN and the lowest in the SLN and intermediate in the GR/A and C3H/He mice. The number of HAN of the glands with no expression of EGF-R mRNA was low compared to that of the glands with EGF-R mRNA expression; 1.3 ± 0.3 vs. $8, 2$ vs. 6.0 ± 0.7 and 2 vs. 12 ± 1.2 in SLN, GR/A and C3H/He, respectively. The mean area of the HAN was significantly lower in the SLN than in the other strains.

TGF α mRNA and EGF-R mRNA expression

The results in normal glands are shown in Table 2 and Fig. 1. At 2 months of age, all samples from each strain expressed TGF α mRNA, and EGF-R mRNA expression was found in all samples from the SHN, GR/A and C3H/He mice, and in 2 of the 4 samples from SLN mice.

At 10 months, all samples from the SHN, SLN and C3H/He mice and 4 of the 5 samples from GR/A mice expressed

Table 1. Normal and preneoplastic mammary gland growth in four strains of virgin mice (Mean \pm SEM)

	Number of mice	Normal gland		HAN	
		Rating	Area (mm ²)	Number	Area (mm ²)
2 months					
SHN	5	3.0 ± 0.3^a	175 ± 13		
SLN	4	1.8 ± 0.5^b	195 ± 11		
GR/A	5	3.1 ± 0.2^a	173 ± 17		
C3H/He	5	3.1 ± 0.5^a	194 ± 18		
10 months					
SHN	6	2.8 ± 0.3^a	227 ± 17	18.2 ± 2.9^a	1.05 ± 0.13^a (109)*
SLN	5	1.8 ± 0.3^b	247 ± 8	3.8 ± 1.6^c	0.54 ± 0.06^b (19)
GR/A	5	1.8 ± 0.3^b	220 ± 21	5.2 ± 0.9^c	1.00 ± 0.13^a (26)
C3H/He	5	$2.4 \pm 0.3^{a,b}$	249 ± 34	10.0 ± 2.1^b	0.90 ± 0.08^a (50)

^{a, b, c} Values with different superscripts are significantly different at $P < 0.05$.

* Number of HAN measured.

Table 2. Frequency of mammary gland expressions of TGF α and EGF-R mRNA in four strains of virgin mice

	2 months		10 months	
	TGF α	EGF-R	TGF α	EGF-R
SHN	6/6 ¹	6/6	6/6	6/6 ^a
SLN	4/4	2/4	5/5	2/5 ^b
GR/A	5/5	5/5	4/5	4/5 ^{a, b}
C3H/He	5/5	5/5	5/5	4/5 ^{a, b}

¹ Number of positive samples/Total number of samples examined.

^{a, b} Values with different superscripts are significantly different at $P < 0.05$.

Table 3. Frequency of mammary tumor expression of TGF α mRNA and EGF-R mRNA in four strains of retired mice

	SHN	SLN	GR/A	C3H/He
TGF α	5/5 ¹	3/3	3/3	5/5
EGF-R	5/5	3/3	3/3	5/5

¹ Number of positive samples/Total number of samples examined.

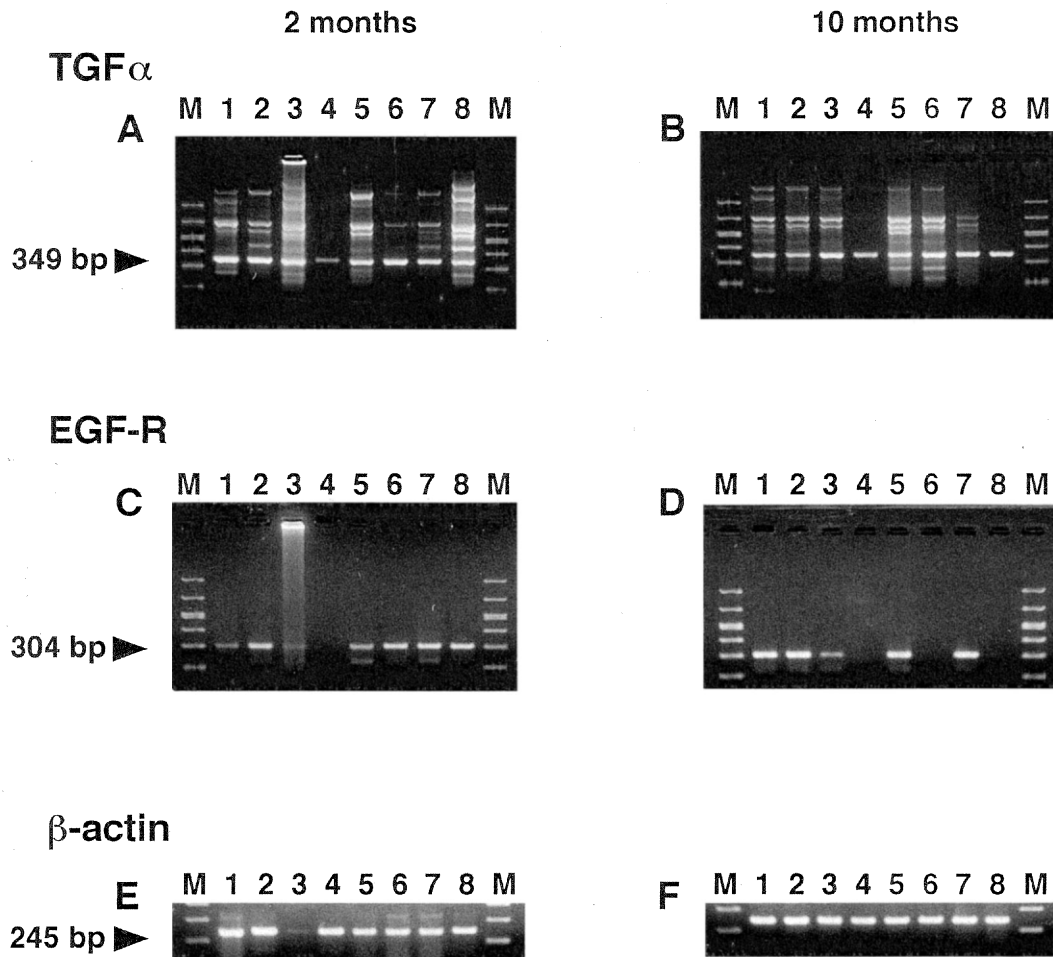


Fig. 1. Agarose gel electrophoresis of RT-PCR products in four strains of virgin mice. The PCR products of the target sequences for TGF α , EGF-R and β -actin were 349 bp, 304 bp and 245 bp, respectively. Lane M, Molecular size of marker; Lanes 1 and 2, SHN; Lanes 3 and 4, SLN; Lanes 5 and 6, GR/A; Lanes 7 and 8, C3H/He.

TGF α mRNA. EGF-R mRNA expression was found in all samples from the SHN mice and in 2 of the 5 samples from SLN and 4 of the 5 from the GR/A and C3H/He mice. The difference in EGF-R mRNA expression between the SHN and SLN mice was significant.

As shown in Table 3, all mammary tumor samples from each strain expressed both TGF α mRNA and EGF-R mRNA at 10 months of age.

DISCUSSION

At 2 months of age, when the mammary glands of the mice consisted mostly of normal tissue without HAN, TGF α mRNA expression was seen in all samples from all four strains of virgin mice. BALB/c and ICR virgin mice, which have no mammary tumor potentials, were also shown to express TGF α mRNA in the mammary glands at about 1.8-3 months of age

(Snedeker *et al.*, 1991; Yasuda *et al.*, 1996). These results suggest that TGF α mRNA expression does not directly relate to whether or not the mouse strain has a mammary tumor potential, and that TGF α participates mostly in normal gland growth at these ages.

EGF-R mRNA expression was also seen in all of the samples from SHN, GR/A and C3H/He mice, but in only 2 of the 4 samples from SLN mice. The mammary glands of the SLN mice consisted mostly of ducts with no end buds, while those of the other 3 strains contained several end-buds. Snedeker *et al.* (1991) reported the participation of EGF-R in the end-bud formation in ovariectomized virgin BALB/c mice at about 3 months of age. Taken together, these findings suggest that the EGF-R at the developmental stage of mammary gland acts specifically on mammary end-bud formation with little relationship to whether the mouse has a mammary tumor potential.

At 10 months, TGF α mRNA expression was seen in almost all mammary gland samples from the four strains of virgin mice examined. Only 1 of the 7 samples from ICR virgin mice expressed TGF α mRNA at about 7 months of age (Yasuda *et al.*, 1996). Thus, the mammary gland expression of TGF α mRNA in aging mice is thought to be higher in strains with a mammary tumor potential than in those without it.

EGF-R mRNA expression was also observed in all of the samples from the SHN mice, but in only 2, 4 and 4 out of 5 samples from the SLN, GR/A and C3H/He mice, respectively. The mammary glands in all strains at 10 months contained preneoplastic HAN. However, in the 3 samples from SLN mice and 1 each from GR/A and C3H/He mice, in which mammary EGF-R mRNA expression was not detected, HAN formation was low compared to the mammary glands with positive expression. This result suggests that EGF-R mRNA is one of the enhancing factors for the formation of HAN and/or its transformation into the tumor.

All of the mammary tumor samples from the four strains showed positive TGF α mRNA expression in this study. This finding is in good agreement with our previous study (Harigaya *et al.*, 1994b). Furthermore, EGF-R mRNA was also detected in all samples examined. This may reflect the hormone independency or autonomy even in the early stage of progression and the marked malignancy of these mouse mammary tumors. It has been reported that in human mammary tumors, EGF-R expression is inversely related to hormone dependency and is thus a sign of poor prognosis (Pimentel, 1994a; Dickson and Lippman, 1995).

ACKNOWLEDGMENTS

We thank Prof. T Harigaya, Laboratory of Functional Anatomy of this University, for his help and interest. This work was supported by Faculty Collaborative Research Grant, Institute of Science and Technology, Meiji University.

REFERENCES

- Alonso S, Minty A, Bourlet Y, Buckingham M (1986) Comparison of three actin-coding sequences in the mouse; Evolutionary relationships between the actin genes of warm-blooded vertebrates. *J Mol Evol* 23: 11–22
- Avivi A, Lax I, Ullrich A, Schlessinger J, Givol D, Morse B (1991) Comparison of EGF receptor sequences as a guide to study the ligand binding site. *Oncogene* 6: 673–676
- Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159
- Derynck R (1988) Transforming growth factor α . *Cell* 54: 593–595
- Derynck R, Roberts AB, Winkler ME, Chen EY, Goeddel DV (1984) Human transforming growth factor- α : precursor structure and expression in *E. Coli*. *Cell* 38: 287–297
- Dickson RB, Lippman ME (1995) Growth factors in breast cancer. *Endocrine Rev* 16: 559–589
- Ethier SP (1995) Growth factor synthesis and human breast cancer progression. *J Natl Cancer Inst* 87: 964–973
- Halter SA, Dempsey P, Matsui Y, Stokes MK, Graves-Deal R, Hogan BLM, Coffey RJ (1992) Distinctive patterns of hyperplasia in transgenic mice with mouse mammary tumor virus transforming growth factor- α . Characterization of mammary gland and skin proliferations. *Amer J Pathol* 140: 1131–1146
- Harigaya T, Tsunoda S, Mizuno M, Nagasawa H (1994a) Different gene expression of mouse transforming growth factor α between pregnant mammary glands and mammary tumors in C3H/He mice. *Zool Sci* 11: 625–627
- Harigaya T, Tsunoda S, Yokoyama H, Yamamoto K, Nagasawa H (1994b) Mouse TGF α gene expression in normal and neoplastic mammary glands and uteri of four strains of mice with different potentials for mammary gland growth and uterine adenomyosis. *Zool Sci* 11: 841–846
- Kenney NJ, Dickson RB (1996) Growth factor and sex steroid interactions in breast cancer. *J Mam Gland Biol Neoplasia* 1: 189–198
- Lee DC, Rose TM, Webb NR, Todaro GJ (1985) Cloning and sequence analysis of a cDNA for rat transforming growth factor- α . *Nature (London)* 313: 489–491
- Luetke NC, Phillips HK, Qiu TH, Copelands NG, Earp SH, Jenkins NA, Lee DC (1994) The mouse *waved-2* phenotype results from a point mutation in the EGF receptor tyrosine kinase. *Genes Develop* 8: 399–413
- Matsui Y, Halter SA, Holt JT, Hogan BLM, Coffey RJ (1990) Development of mammary hyperplasia and neoplasia in MMTV-TGF α transgenic mice. *Cell* 61: 1147–1155
- Mizuno M, Yamamoto K, Sakamoto S, Mori T, Harigaya T, Nagasawa H (1994) Normal and neoplastic mammary gland growth in MMTV/TGF α transgenic mice. *in vivo* 8: 263–270
- Mühlbock O (1965) Note on a new inbred mouse-strain GR/A. *Europ J Cancer* 1: 123–124
- Nagasawa H, Yanai R, Taniguchi H, Tokuzen R, Nakahara W (1976) Two-way selection of a basal stock of Swiss albino mice for mammary tumorigenesis: Establishment of two new strains (SHN and SLN). *J Natl Cancer Inst* 57: 425–430
- Nagasawa H, Yanai R, Taniguchi H, Hayashi H (1979) Mammary tumor incidence in relation to the pattern of oestrous cycles in mice. *Hormone Res* 10: 123–129
- Nagasawa H, Yanai R, Nakajima H, Namiki H, Kikuyama H, Shiota K (1980) Inhibitory effects of potassium thiocyanate on normal and neoplastic mammary development in female mice. *Europ J Cancer* 16: 473–480
- Nagasawa H, Konishi R, Naito T, Ohmiya S, Mori T (1987a) Relationship between mammary tumorigenesis and uterine adenomyosis in four strains of mice. *in vivo* 1: 237–240
- Nagasawa H, Naito T, Namiki H (1987b) Mammary gland growth and

- serum levels of prolactin and growth hormone in four strains of male mice comparison with females. *Life Sci* 40: 1473–1478
- Paria BC, Das SK, Andrews GK, Day SK (1993) Expression of the epidermal growth factor receptor gene is regulated in mouse blastocysts during delayed implantation. *Proc Natl Acad Sci USA* 90: 55–59
- Pimentel E (1994a) Epidermal growth factor. In "Handbook of Growth Factors Vol 2" CRC Press, Boca Raton, pp 97–185
- Pimentel E (1994b) Transforming growth factors. In "Handbook of Growth Factors Vol 2" CRC Press, Boca Raton, pp 263–350
- Snedeker SM, Brown CF, Di Augustine RP (1991) Expression and functional properties of transforming growth factor- α and epidermal growth factor during mouse mammary gland ductal morphogenesis. *Proc Natl Acad Sci USA* 88: 276–280
- Staats J (1985) Standardized nomenclature for inbred strains of mice. 8th listing. *Cancer Res* 45: 945–977
- Todaro GJ, Fryling C, DeLarco JE (1980) Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. *Proc Natl Acad Sci USA* 77: 5258–5262
- Vaughan TJ, Pascall JC, Brown KD (1992) Nucleotide sequence and tissue distribution of mouse transforming growth factor- α . *Biochim Biophys Acta* 1132: 322–324
- Yanai R, Nagasawa H (1978) Development and growth of pregnancy-dependent and -independent mammary tumors in GR/A strain of mice and their interrelationship. *Gann (Japanese Journal of Cancer Research)* 69: 25–30
- Yasuda M, Tanaka M, Nagasawa H (1996) Comparison of mammary gland growth and some physiological parameters in a high mammary tumor strain of SHN mice and ICR, most popular and 'normal' mice. *Exp Anim* 45: 141–148

(Received March 17, 1997 / Accepted June 10, 1997)