Search for Mouse Mammary Tumor Virus-like *env* Sequences in Cancer and Normal Breast from the Same Individuals[1]

Stella M. Melana, James F. Holland, and Beatriz G-T. Pogo[2]

Departments of Medicine [S. M. M., J. F. H., B. G. T. P.] and Microbiology [B. G-T. P.], Mount Sinai School of Medicine, New York, New York 10029

ABSTRACT

We have reported previously that a 660-bp sequence homologous to the *env* gene of the mouse mammary tumor virus, but not to the known endogenous retroviruses, was present in 38% of human breast cancers (Y. Wang *et al.*, Cancer Res., *55:* 5173–5179, 1995). A unique 250-bp internal sequence was equally present in formalin-fixed breast carcinoma. It was not detected in normal human breasts or in other tumors. In this study, we have investigated whether this 250-bp *env* sequence was also present in the formalin-fixed normal tissues of individuals with *env* sequence-positive breast cancer. Separate paraffin-embedded sections from breast carcinoma and normal breast tissues from the same individual were obtained from the Cooperative Breast Cancer Tissue Registry of the National Cancer Institute. The 250-bp *env* sequence was detected in 30.1% of the 106 tumors but in only 1 of the 106 normal breast tissues. These results indicate that the sequence is absent in normal tissues and thus is not genetically transmitted. This strongly implies that it is of exogenous origin.

INTRODUCTION

Evidence that an agent similar to MMTV[3] may be involved in the development of human breast cancer has been controversial (as discussed in Refs. 1 and 2). Part of the problem is the presence of HERs in the human genome that hamper the detection of exogenous viruses. One family of these HERs, the HERV-Ks, has an overall 50% homology to MMTV (3, 4). To overcome the problem caused by the presence of endogenous sequences, we have selected regions of the MMTV genome with very low homology to the HERV-K10, the best-characterized member of the HERV-K family. We have searched for these MMTV-like sequences in a large panel of human breast cancers using PCR and specific MMTV primers. We found that a 660-bp sequence of the *env* gene with 90–98% homology to MMTV was present in 38% of unselected breast cancers and 1.4% of 140 normal breast specimens obtained from reduction mammoplasties (5). An internal 250-bp sequence was detected in 36% of paraffin-embedded specimens (5). Similar results have been reported recently by Etkind *et al.* (6). The MMTV-like sequences were found to be expressed in 66% of the tumors that contain them and in certain breast cancer cell lines (7).

In a collaborative study with researchers of the Istituto Nazionale per lo Studio e la Cura dei Tumori (Milan, Italy), we detected the presence of the *env* gene sequence in 37.7% of 70 paraffin-embedded Italian breast cancer samples analyzed (8). The presence of the sequence was not significantly associated with any of the clinical, pathological, or biological parameters studied. It did correlate, however, with expression of laminin receptor, a marker for invasiveness and poor prognosis (8).

We have now investigated the presence of the *env* sequence in breast cancer and normal breast tissues from the same individuals. Paired specimens were provided by the CBCTR, which is funded by the National Cancer Institute. The CBCTR also provides information about other clinical and pathological parameters not previously studied. The purpose of this study was to address the fundamental question of whether the *env* sequence was also present in the normal tissues of an individual with *env* sequence-positive breast cancer, which could be critical in establishing endogenous or exogenous origin.

MATERIALS AND METHODS

Paraffin-embedded sections of 106 paired tumors and normal breasts obtained at the time of primary surgery were provided by the CBCTR. Sections were cut with disposable blades that were changed for each individual sample. All equipment used was wiped with 10% sodium hypochloride solution. DNA was extracted from two 5-μm or one 10-μm sections following the conditions described in previous publications (5, 8). DNA was purified using the 625 column from Eppendorf Co. PCR was carried out using primers 2N and 3N. Hybridization was carried out with ^{32}P-labeled oligonucleotide 2aN (5, 8). Sequences for primers were as follows: (*a*) 2N, 5′-CCTACATCTGCCTGTGTTAC (positions 1386–1405); (*b*) 3N, 5′-ATCTGTGGCATACCTAAAGG (positions 1640–1621); and (*c*) 2aN, 5′-CCGTACGTGCTGCTACCTGTA (positions 1557–1577). The amplification reaction was carried out with 100 pmol of primers using PCR BEADS from Amersham Pharmacia Biotech (Buckinghamshire, United Kingdom). Each reaction contained, in 25 μl, the following components: (*a*) 1.5 units of Taq DNA polymerase; (*b*) 10 mM Tris-HCl (pH 9.0 at room temperature); (*c*) 50 mM KCl; (*d*) 1.5 mM $MgCl_2$; and (*e*) 200 mM of each of the four nucleotides. Thermocycling was performed in a DNA cycler (Perkin-Elmer) by denaturation at 94°C for 1–5 min, annealing at 55°C for 1 min, and elongation at 72°C for 1–5 min for 35 cycles. Each sample was tested three times to assure reproducibility of the reaction.

The DNA quality was tested by amplifying a 260-bp sequence of the β-globin gene using primers GH-20 (5′-GAAGAGCCAAGGACAGGTAC) and PCO4 (5′-CAACTTCATC-

Received 8/21/00; revised 11/27/00; accepted 11/28/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by the T. J. Martell Foundation for Leukemia, Cancer and AIDS Research; The Kash Family Foundation; The Jane Grinberg Research Fund; The Chemotherapy Foundation; The Ellen Block Memorial Fund; and The Myra Shaw Anti-Cancer League.

[2] To whom requests for reprints should be addressed, at Departments of Microbiology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029. Phone : (212) 241-8822; Fax: (212) 828-4194; E-mail: pogob01@doc.mssm.edu.

[3] The abbreviations used are: MMTV, mouse mammary tumor virus; HER, human endogenous retrovirus; CBCTR, Cooperative Breast Cancer Tissue Registry.

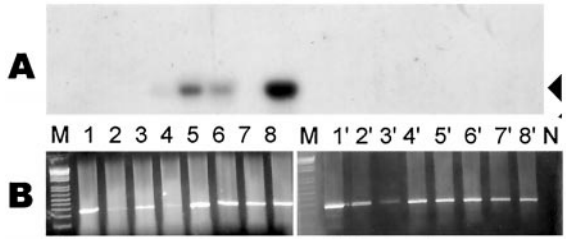

Fig. 1 Amplification of 250 bp of the MMTV-like *env* gene. DNA was extracted separately from paraffin-embedded breast carcinomas and normal breast tissues from the same individual. PCR was performed using primers 2N and 3N, and Southern blot hybridization was performed using 5′ ^{32}P-labeled 2aN as a probe. *A*, *Lanes 1–8*, breast carcinomas; *Lane M*, molecular weight marker; *Lanes 1′–8′*, corresponding normal breast tissue samples. *Lanes 4*, *5*, *6*, and *8* demonstrate *env*-positive tumors. *Arrow* indicates the position of the 250 bp in the gel. *B*, β-globin amplification. Samples were as described in *A*. *Lane N*, negative control.

CACGTTCACC). Cycling conditions were 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min for 40 cycles (9).

RESULTS

The presence of the 250-bp *env* sequences was studied in DNAs that had first been tested for DNA quality control by amplification of a 260-bp sequence of the β-globin gene, as shown in Fig. 1*B*. Of 106 tumor and normal breast samples studied, 32 were *env* positive. The results are summarized in Table 1. Only one of the normal samples gave a positive signal. However, the corresponding tumor was negative. A typical Southern blot result showing four positive breast cancer samples and the corresponding normal breast samples is represented in Fig. 1*A*.

DISCUSSION

In this study, the presence of *env* sequences in breast tumors obtained from different parts of the country was almost 30%, slightly lower than the 36% reported in previous studies using paraffin-embedded tissues (5). The results reported here clearly indicated that the normal breasts of individuals with *env*-positive breast cancers do not contain the sequence. In three independent PCR determinations, only one normal breast sample gave a positive signal. The test for potential plasmid contamination was negative. Microscopic examination of the remaining nonextracted slides of this specimen revealed that they contained only normal tissue. However, the breast cancer from this individual was negative. Therefore, we have concluded that the sample was contaminated with positive cancer tissue or DNA either during tissue sectioning or DNA extraction, despite all of the precautions taken (5).

Previously published results indicate that four normal breasts and seven mononuclear blood cells from patients with *env*-positive breast tumors were *env* negative (5). In the present report, a large number of breast cancer samples were studied with their corresponding normal tissues.

The origin of the viral sequence has been discussed extensively (5, 7). Because it has been found almost exclusively in breast tumors, it has been suggested that it has an exogenous origin (5, 6). The possibility of an endogenous sequence was considered highly unlikely because this sequence has very low homology to the *env* genes of known endogenous viruses HERV-K10 and HERV-K-T47D (3, 4).

The postulation that this sequence represents an unknown endogenous virus amplified in tumors and therefore only detectable in malignant tissues is also improbable because the sequence was found in euploid as well as aneuploid tumors (8). If aneuploidy were the cause, other human cancers should be positive for the sequence as well. Furthermore, an endogenous sequence should at least be present in one copy/cell and should be easily detectable by amplification in normal tissues. Such was not the case.

Table 1 Detection of MMTV *env* gene-like sequences in carcinoma and normal breast tissue DNA

Sample	*N*	*env*+	%
Carcinoma	106	32	30.10
Normal	106	1	0.94

The results presented herein strongly imply that the *env* gene sequences present in breast cancers are of exogenous origin. Epidemiological inference for the participation of MMTV in human breast cancer has been published previously (10). Moreover, we have recently detected the entire proviral structure in two fresh breast cancers with 95% overall homology to MMTV and low homology to HERVK-10.[4] How these sequences are involved in the pathogenesis of breast cancer is under intensive investigation.

REFERENCES

1. Pogo, B. G-T., and Holland, J. F. Possibilities of a viral etiology for human breast cancer: a review. Biol. Trace Elem. Res., *56:* 131–142, 1997.
2. Keydar, I. Retroviruses in breast cancer. J. Women's Cancer, *1:* 1–7, 1998.
3. Ono, M., Yasunaga, T., Miyata, T., and Ushikubo, H. Nucleotide sequence of human endogenous retrovirus genome related to the mouse mammary tumor virus genome. J. Virol., *60:* 589–598, 1986.
4. Seifarth, W., Baust, C., Murr, A., Skladny, H., Krieg-Schnmeider, F., Blusch, J., Werner, T., Hehlmann, R., and Leib-Mosch, C. Proviral structure, chromosomal location and expression of HERV-K-T47D, a novel human endogenous retrovirus derived from T47D particles. J. Virol., *72:* 8384–8391, 1998.
5. Wang, Y., Holland, J. F., Bleiweiss, I. J., Melana, S., Liu, X., Pelisson, I., Cantarella, A., Stellrecht, K., Mani, S., and Pogo, B. G-T. Detection of mammary tumor virus *env* gene-like sequences in human breast cancer. Cancer Res., *55:* 5173–5179, 1995.
6. Etkind, P., Du, J., Khan, A., Pillitteri, J., and Wiernik, P. H. Mouse mammary tumor virus-like *env* gene sequences in human breast tumors and in a lymphoma of a breast cancer patient. Clin. Cancer Res., *6:* 1273–1278, 2000.
7. Wang, Y., Go, V., Holland, J. F., Melana, S. M., and Pogo, B. G-T. Expression of MMTV-like *env* gene sequences in human breast cancer. Clin. Cancer Res., *4:* 2565–2568, 1998.
8. Pogo, B. G-T., Melana, S. M., Holland, J. F., Mandeli, J. F., Piloti, S., Casalini, P., and Ménard, S. Sequences homologous to the MMTV *env* gene in human breast carcinoma correlate with overexpression of laminin receptor. Clin. Cancer Res., *5:* 2108–2111, 1999.
9. Resnick, R. M., Cornelissen, M. T. E., Wright, D. K., Eichinger, G. H., Fox, H. S., Schegget, J. T., and Manos, M. M. Detection and typing of human papillomavirus in archival cervical cancer specimens by DNA amplification with consensus primers. J. Natl. Cancer Inst. (Bethesda), *82:* 1477–1484, 1990.
10. Stewart, T. H. M., Sage, R. D., Stewart, A. F. R., and Cameron, D.W. Breast cancer incidence highest in the range of one species of house mouse, *Mus domesticus*. Br. J. Cancer, *82:* 446–451, 2000.

[4] B. Liu, Y. Wang, S. M. Melana, I. Pelisson, V. Najfeld, J. F. Holland, and B. G-T. Pogo. Identification of a proviral structure in human breast cancer, Cancer Res., in press, 2001.