Search for Mouse Mammary Tumor Virus-like \textit{env} Sequences in Cancer and Normal Breast from the Same Individuals\textsuperscript{1}

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ABSTRACT

We have reported previously that a 660-bp sequence homologous to the \textit{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 \textit{env} sequence was also present in the formalin-fixed normal tissues of individuals with \textit{env} sequence-positive breast cancer. Separate \textit{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 \textit{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\textsuperscript{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 \textit{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 \textit{paraffin-embedded} specimens (5). Similar results have been reported recently by Etkind \textit{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 \textit{env} gene sequence in 37.7\% of 70 \textit{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 \textit{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 \textit{env} sequence was also present in the normal tissues of an individual with \textit{env} sequence-positive breast cancer, which could be critical in establishing endogenous or exogenous origin.

MATERIALS AND METHODS

\textit{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-\textmu m or one 10-\textmu m 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 \textsuperscript{32}P-labeled oligonucleotide 2aN (5, 8).

Sequences for primers were as follows: (a) 2N, 5'-CCTACATC-TGGCGTTGTTAC (positions 1386–1405); (b) 3N, 5'-ATCTG-TGGCATCCAAGG (positions 1640–1621); and (c) 2aN, 5'-CGTACGTGCTGCTACCTGTA (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 25 \textmu l, the following components: (a) 1.5 units of Taq DNA polymerase; (b) 10 mM Tris-HCl (\textit{pH} 9.0 at room temperature); (c) 50 mM KCl; (d) 1.5 mM MgCl\textsubscript{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 \textbeta-globin gene using primers GH-20 (5'-GAA-GAGGACAAGGACAGGTAC) and PC04 (5'-CAACTTCATC-

\textsuperscript{1} The abbreviations used are: MMTV, mouse mammary tumor virus; HER, human endogenous retrovirus; CBCTR, Cooperative Breast Cancer Tissue Registry.

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normal breast samples is represented in Fig. 1 ing four positive breast cancer samples and the corresponding env were in Fig. 1. Of 106 tumor and normal breast samples studied, 32 B amplification of a 260-bp sequence of the DNAs that had first been tested for DNA quality control by DISCUSSION 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. 1B. 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. 1A.

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.

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


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