Mouse Mammary Tumor Virus-like Gene Sequences in Breast Tumors of Australian and Vietnamese Women

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ABSTRACT

Purpose: There is considerable evidence that the presence of mouse mammary tumor virus (MMTV)-like gene sequences in human breast cancer is highly associated with human breast carcinoma. Previous studies have found MMTV-like gene sequences in 38% of breast cancer tissue from United States women. The prevalence of these sequences in Australian and Vietnamese women has never been reported.

Experimental Design: Using PCR and primers that amplify MMTV-like gene sequences, we tested cancerous and benign breast tissue from Caucasian-Australian, Vietnamese-Australian, and Vietnamese women.

Results: MMTV-like gene sequences were amplified in 19 of 45 (42.2%) archival breast cancer biopsy tissues from Caucasian-Australian women, but only 1 of 120 (0.8%) and 0 of 41 breast cancer biopsy tissues from Vietnamese and Vietnamese-Australian women, respectively. The same sequences were found in only 2 of 111 (1.8%) and 0 of 60 normal (benign) breast tissue samples from Australian and Vietnamese women, respectively.

Conclusions: MMTV-like gene sequences are found in only some human populations and are rarely found in normal human breast tissue from all populations, suggesting they are not present in the normal human genome and have been acquired.

INTRODUCTION

There is substantial indirect and limited direct evidence that a similar retrovirus to MMTV, with additional cofactors such as diet and hormones, may influence human breast carcinogenesis (1–4). The prevalence of MMTV-like gene sequences is high in North American and Italian women (3, 5). The prevalence of these MMTV-like gene sequences in Australian and Vietnamese populations has never been reported; however, it has been hypothesized that prevalence levels in Australian women would be similar to those reported in North American women (6). Vietnamese are an ethnically distinct group where prevalence of these sequences is unknown. Breast cancer is the most common malignancy in Australian women with an age standardized incidence rate of 101.3 cases per 100,000 population (7). In Vietnam, breast cancer is the second most common malignancy, after cervical cancer, with an age standardized incidence of 12.2 cases per 100,000 (8). In this study we have assessed the prevalence of MMTV-like gene sequences in invasive breast cancer tissues from Australian and Vietnamese women both in Vietnam and Australia, and compared these with rates in normal breast tissue from the same populations.

MATERIALS AND METHODS

Unselected breast specimens were obtained from archival material from women who had undergone surgery for breast cancer. Specimens were obtained from 120 Vietnamese subjects who were treated at the National Cancer Hospital in Hanoi, Vietnam, and 41 specimens from Vietnamese-Australian and 45 specimens from Caucasian-Australian subjects who were treated in Sydney, Australia. Archival breast tissue was also obtained from 60 Vietnamese and 111 Australian women who had benign breast conditions, and the age of each woman was recorded. All of the specimens were fixed in 10% formalin, mounted in paraffin blocks, and 10-μm sections cut using a microtome. The blade was cleaned systematically with alcohol between each sample to prevent cross-contamination, which was additionally avoided by recording the order of cutting and repeating any adjacent PCR-positive samples.

Breast tumors were diagnosed according to standard criteria (9). Cancerous epithelial breast cells were identified using H&E stains (10). Human breast tissues were processed in a class II laminar flow hood, located in a separate laboratory to where PCR was performed. Paraffin was removed from tissues with two washes of xylene, supernatant removed, and the pellet washed in 100% ethanol. Tissues were resuspended in 100 μl of digestion buffer (150 mM NaCl, 15 mM Tris-HCl, 1 mM EDTA, and 0.1% SDS) with 5 μl of protease K (20 mg/ml), incubated at 55°C for 3 h, and then at 95°C for 10 min to inactivate the...
proteinase K. Phenol-chloroform extraction was performed, the DNA precipitated in ethanol, and the supernatant removed. The pellet was dried and resuspended in 20 μl of 10 mM Tris (pH 8)-1 mM EDTA buffer containing RNase, and the DNA concentration determined using a spectrophotometer.

Nested PCR was performed using a Perkin-Elmer 9700 thermal cycler and Taq polymerase (Promega) with minor modifications to published techniques, and primers using standard precautions and controls (3, 4, 11). Positive controls consisted of DNA extracted from the MCF-7 human breast cancer cell line that expresses MMTV env gene-like sequences (4), and DNA quality was assessed by amplifying extracted DNA with p53 and β-globin primers (12, 13). Cycle conditions for MMTV outer PCR with primers 5L and 3L (4) were 94°C for 2 min, 30 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 40 s, followed by 72°C for 3 min. Ten μl of outer product was then added to the inner reaction mix using primers 1X and 2NR (4). PCR was performed at 94°C for 2 min, 30 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, followed by 72°C for 3 min. PCR products were analyzed using agarose gel electrophoresis, and all of the positive PCR results for MMTV-like gene sequences were repeated. PCR products were sequenced and sequences analyzed as described previously (14).

Power calculations on sample size were performed using standard calculations (15), and the association between MMTV-like gene sequences and breast cancer assessed using Fisher’s exact test.

RESULTS

PCR screening of 45 breast cancer samples from Australian women and 120 from Vietnamese women showed the prevalence of MMTV-like gene sequences of 42.2% (19 of 45) and 0.8% (1 of 120), respectively (Fig. 1; Table 1), a difference that was statistically significant (P = 0.0001). All 41 of the breast cancer tissues from first generation Vietnamese-Australian women were negative for MMTV-like gene sequences. Normal breast tissue from 111 Australian and 60 Vietnamese women were tested using nested PCR to detect MMTV-like gene sequences as control subjects for each PCR reaction. All of the Vietnamese benign breast tissue samples were negative using PCR, and only 2 of 111 (1.8%) normal breast tissues from Australian women who had reduction mammoplasties were positive for MMTV-like gene sequences (Table 1). Both of these women had no clinical evidence of breast cancer.

PCR results were correlated with the severity of cancer and classified histopathologically as normal, ductal carcinoma in situ, or infiltrating ductal carcinoma. The grade of cancer correlated closely with the presence of MMTV-like gene sequences. That is, the prevalence of these viral sequences increased with severity of cancer (Table 1), with 2 of 111 (1.8%) normal breast tissue samples, 5 of 19 (26.3%) ductal carcinoma in situ samples, and 14 of 26 (53.8%) infiltrating ductal carcinoma samples positive for MMTV-like gene sequences. Amplicons from all 19 of the positive Australian and 1 positive Vietnamese breast cancer sample were sequenced and aligned with the env region of MMTV. Sequence alignments demonstrated that the 20 isolates were highly homologous to the env region of MMTV, with between 97 and 99% identity at the nucleotide level (GenBank accession nos. AY161328–AY161347).

DISCUSSION

In this study we have shown that the prevalence of MMTV-like nucleotide sequences in invasive breast cancer tissues from Australian women with breast cancer is much higher than in normal (benign) breast tissue and much higher than in Vietnamese women (both in Vietnam and Australia) with breast cancer (0.8% and 0%, respectively). This specific env gene sequence has been identified previously in 38% of breast cancers from United States women (4), and confirmed in United States women by Etkind et al. (16) and in Australian women by our group.

A significant difference in the prevalence of antibodies reactive with MMTV and breast cancer rates has been reported between women from Western and Eastern countries (17), with the prevalence of antibodies reactive with MMTV in the sera of women with breast cancer found to vary from <5% in mainland

Table 1 MMTV-like gene sequence rates in breast cancer sections (from women with breast cancer) and benign breast tissue sections (from women without breast cancer) from Australian and Vietnamese women, showing correlation of prevalence of MMTV-like gene sequences with normal, ductal carcinoma in situ, and infiltrating ductal carcinoma Australian breast tissues

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Total no. studied</th>
<th>No. PCR-negative</th>
<th>No. PCR-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer (total)</td>
<td>45</td>
<td>26</td>
<td>19 (42.2%)</td>
</tr>
<tr>
<td>Ductal carcinoma in situ</td>
<td>19</td>
<td>14</td>
<td>5 (26.3%)</td>
</tr>
<tr>
<td>Infiltrating ductal carcinoma</td>
<td>26</td>
<td>12</td>
<td>14 (53.8%)</td>
</tr>
<tr>
<td>Benign breast tissue (control subjects)</td>
<td>111</td>
<td>109</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>Vietnamese-Australian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>41</td>
<td>41</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Vietnamese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>120</td>
<td>119</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Benign breast tissue (control subjects)</td>
<td>60</td>
<td>60</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>
Chinese, 18.6% in North Americans, 38% in Indians, and 61.9% in East African women (17). Antibody levels reported in Chinese women are similar to the extremely low prevalence levels of MMTV-like gene sequences in Vietnamese women as reported in this study (<1%).

It is interesting that the breast cancer tissues from the first generation Vietnamese-Australian women did not contain MMTV-like gene sequences. This is similar to the low levels detected in Vietnamese women from Hanoi, and unlike the high levels reported in Caucasian-Australian women. It would be interesting to examine the prevalence of MMTV-like gene sequences in the next generation of Vietnamese women born in Australia, to additionally investigate the impact of geography and environment on MMTV-like gene sequences and breast cancer.

The differences in breast cancer rates and prevalence of MMTV-like gene sequences between populations may be related to host factors and the geographic distribution of different mouse species (6), as exogenous MMTV is found in up to 50% of *Mus domesticus* from Western countries (18). It has been hypothesized that MMTV may have the ability to infect across species (6, 18). The high prevalence of MMTV-like gene sequences in Australian women (similar to United States women) and low prevalence in Vietnamese women (both in Australia and Vietnam) may support this hypothesis.

Histopathological analysis of the Australian breast tissues screened showed an increasing prevalence of MMTV-like gene sequences with severity of cancer. This suggests that these MMTV-like gene sequences are associated with more invasive breast carcinoma and may be involved in the etiology of human breast cancer.

There is recent evidence that suggests that these MMTV-like gene sequences may be of exogenous origin (19, 20). The finding of MMTV-like gene sequences in breast cancer tissue, but rarely in normal breast tissue, supports this hypothesis.

Our findings confirm that MMTV-like sequences occur in a high proportion of women with breast cancer in some human populations and are rarely found in normal breast tissue. This supports the contention that these sequences represent a virus associated with human breast carcinogenesis and not a normal part of the human genome.

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