Prevention and Epidemiology

Mouse Mammary Tumor Virus–like Sequences in Human Breast Cancer

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Abstract

Mouse mammary tumor virus (MMTV) sequences have been reported to be present in some human breast cancers, but it is unclear whether they have any causal role. In mice, MMTV promotes tumor formation indirectly by insertional mutagenesis of Wnt oncogenes that lead to their activation. In this study, we investigated the status of Wnt-1 in human breast cancers harboring MMTV-like sequences encoding viral envelope (env) genes. We confirmed the detection of env sequences in the nucleus of human breast cancer specimens that are similar in appearance to mouse mammary tumors expressing MMTV env sequences. MMTV env sequences in human breast cancers were also nearly indistinguishable from env sequences in mouse MMTV isolates. Further, Wnt-1 expression was higher in specimens of env-positive ductal carcinoma in situ and invasive ductal carcinoma, relative to env-negative specimens. Our findings extend the evidence that MMTV sequences found in naturally occurring mouse mammary tumors can be found in some human breast cancers, prompting further evaluation of causal roles in these settings. Cancer Res; 70(9); 3576–85. ©2010 AACR.

Introduction

Mouse mammary tumor virus (MMTV)–like virus has been a major suspect as a cause of some human breast cancers for over 50 years (1). Despite the substantial evidence that MMTV-like virus may have a role in human breast cancer, the development of conclusive evidence has been elusive (2). The reasons include the difficulty in detecting the low levels of MMTV in human breast cancers and concern that the main investigative tool (PCR) may be confounded by false-positive and false-negative results due to sequence variations that affect primer or probe binding (3). The purpose of this study is to consider and confirm the existing evidence and to conduct additional investigations aimed at determining whether MMTV has a role in some human breast cancers.

MMTV-like virus envelope (env) gene sequences have been identified in breast cancer specimens from women in seven countries—Australia, Argentina, China, Italy, Mexico, Tunisia, and United States—but rarely in normal breast specimens (2). Seventy percent of the complete MMTV-like virus genome, identified in human breast cancer specimens and viral particles from human breast metastases, have been sequenced and shown to display 91% to 99% homology to MMTV from mouse mammary tumors (4–6). In a recent study, env and long terminal repeat sequences with >98% homology to those of MMTV have been identified in breast cancers that had occurred in a mother, father, and daughter of the same family, living under the same roof (7). MMTV can infect, integrate, and multiply in human breast epithelial cancer cell lines (8–10).

MMTV is strongly associated with the development of mouse mammary tumors in both wild and inbred mice. MMTV may be transmitted endogenously through the germ-line or exogenously through the mother’s milk to newborn pups; MMTV virions (intact fully formed replicable but non-integrated viruses) are ingested into the gut and enter the lymphatic system through lymphocytes and dendritic cells in the Peyer’s patches; MMTV-infected lymphocytes move to the spleen where they remain dormant for long periods then possibly during mouse puberty; and the infected lymphocytes move to the mammary glands where the MMTV integrates into the DNA of the host mammary epithelial cells (11). Although the integration of MMTV proviral DNA is thought to be essentially random, integration of an MMTV provirus in the vicinity of a number of host oncogenes, particularly near the Wnt and Fgf family genes, results in inappropriate oncogene expression and clonal outgrowth of the infected cell (reviewed in refs. 11–13). When abnormally expressed in mouse

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

In this article, we have followed the convention that malignancies of the human breast are referred to as breast cancer or breast tumors and malignancies of the mouse breast are referred to as mammary tumors.

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mammary tissues, Wnt-1 contributes to hyperplasia and malignant progression (11).

The influence of hormones on the MMTV virus is of special interest because of the dependency of human breast cancer on estrogens and other hormones (14). Estrogens induce mouse mammary tumors in the presence of MMTV but not in its absence (15). Between 10 to 100 times more virus is produced by corticoid-influenced MMTV-containing cells than by controls (16). In mice mammary tumors, integration in the vicinity of the Wnt (int) loci occurs as an early event and the (clonal) growth and the development of the tumor is initially hormone dependent. The tumors become hormone independent and progress presumably because of the accumulation of other genetic insults (17). This is particularly noteworthy as the prevalence of MMTV-like virus sequences in human gestational breast cancer (cancer occurring during pregnancy or 12 months postpartum) is as high as 62% compared with 30% to 38% for sporadic breast cancers, which suggests an influence of hormones on MMTV-like viruses also in humans (18).

Wnt pathways seem to have a role in human breast and other cancers with overexpression of Wnt proteins in up to 60% of breast cancers (19). In an elegant series of experiments, Ayyanan and colleagues (20) showed that increased Wnt-1 expression has an oncogenic influence on normal human breast epithelial cells (obtained from reduction mammoplasties). Specifically, they showed that Wnt-1 increased cell proliferation and these proliferating cells created tumors when injected into immunocompromised mice. These tumors had typical histologic characteristics of medullary carcinoma of the human breast (sheets of cancer cells with infiltrating lymphocytes). The Ayyanan and colleagues (20) studies support the findings by He and colleagues (21) and Wieczorek and colleagues (22) that the inhibition of Wnt-1 signaling by RNA interference and monoclonal anti–Wnt-1 antibody induced the apoptosis of immortalized breast cancer cultured cells. Thus, overexpression of Wnt-1 induced either by the insertion of MMTV in the vicinity of the gene or by mutation of Wnt-1 gene has oncogenic potential.

Recently, it has been shown that in addition to activation of cellular proto-oncogenes such as Wnt-1, MMTV can contribute to mammary tumorigenesis by direct transformation of normal human epithelial cells by expression of signaling proteins (23). Moreover, evidence has been presented that the MMTV env gene participates in mammary epithelial cell transformation in vivo using a transgenic mouse model (24). Thus, there may be more than one mechanism by which MMTV causes mammary tumors in mice.

Based on these findings, we sought to investigate whether (a) the presence of MMTV-like virus env gene sequences in human breast cancer would be associated with high expression of the oncogene Wnt-1, (b) the prevalence of MMTV-like virus env gene sequences would be higher in human breast cancers with similar histologic characteristics to MMTV mouse mammary tumors, and (c) MMTV-like virus env sequences detected in human breast cancer are the same as in mouse mammary tumors.

Materials and Methods

To test these hypotheses, we aimed to (a) confirm the presence and location of MMTV-like virus in human breast cancer specimens by two independent methods: standard and in situ PCR and immunohistochemistry (IHC; against the gp52 env protein), (b) assess the Wnt-1 protein expression levels in tumor sections by IHC, (c) compare the morphologic (histologic) characteristics typical of MMTV-associated mouse mammary tumors and (MMTV associated) human breast cancer, and (d) compare the MMTV env sequences using sequence alignment computer programs (BLAST and CLUSTAL) to determine if MMTV-like virus env sequences identified in human breast cancer, human liver, and mouse mammary tumors in wild and inbred mice were homologous.

For investigations based on standard and in situ PCR and IHC, we used unselected archival formalin-fixed human breast cancer specimens and unselected archival formalin-fixed noncancerous breast specimens from women who had breast reduction surgery. All the archival specimens were from women living in Australia. We also used formalin-fixed MMTV-positive mouse mammary tumors from The Jackson Laboratory.

PCR

Genomic DNA preparation and testing. Previously described protocols were used to extract genomic DNA from the breast cancer specimens (25). The DNA quality was subsequently tested by amplification of a 268-bp fragment of the β-globin gene using HotStarTaq DNA polymerase (Qiagen) and primers G073 (5′-GAAGGCGCAAGCAGGTAC-3′) and G074 (5′-CAACCTCAGCCGTTACCC-3′). The cycling conditions were 95°C, 15 minutes; followed by 35 cycles of 95°C, 30 seconds; 55°C, 30 seconds; 72°C, 1 minute; and a final extension at 72°C, 10 minutes. The amplified products were visualized on 2% agarose gel. All the samples were shown to be suitable for PCR analysis.

Screening for MMTV env sequences. Nested PCR was performed in a total volume of 20 μL using HotStarTaq DNA polymerase (Qiagen) with conditions of 95°C, 15 minutes; followed by 35 cycles of 95°C, 30 seconds; 50°C to 55°C, 30 seconds; 72°C, 1 minute; and a final extension at 72°C, 10 minutes. The primary PCR was performed using outer primers 1N (5′-CCTCAGTGCAGATCGCCT-3′) and 4N (5′-GAATGCTTGCTGCCT-3′) based on Wang and colleagues (26). One microliter of the resulting PCR product was subjected to a further amplification in the secondary PCR using inner primers 2N (5′-CCTACATGTGCCGTGTTAC-3′) and 3N (5′-ATCTGTTGGCATACTAAAGG-3′). The MCF-7 cultured cell line (MMTV env−positive human breast adenocarcinoma) genomic DNA and p203 (containing a cloned MMTV env fragment) plasmid DNA were used as positive controls. Genomic DNA isolated from FFbw011 cells (primary foreskin fibroblasts) or the omission of the DNA template were used as negative controls. The nested PCR was independently repeated for each sample. The amplified products were visualized on 2% agarose gel. When the DNA quality was suitable for PCR analysis, the DNA was visualized on 2% agarose gel. All the samples were shown to be suitable for PCR analysis.

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products were visualized on 2% agarose gels. Eighteen randomly selected positive PCR products were purified by the QIAquick Gel Extraction kit (Qiagen) and sequenced using the ABI Dye terminator sequencing kit and ABI 3730 automated sequencer (Applied Biosystems).

**In situ PCR.** Four-micrometer-thick sections of breast tumor tissue were cut and placed onto silanized slides for *in situ* PCR. Positive controls (virus-positive tissue) and negative controls (virus-negative tissue), controls omitting DIG-11-dUTP (to confirm the incorporation of the DIG label), controls omitting primers (to confirm the signal was the result of specific amplification rather than self-priming of degraded tissues), or controls omitting Taq polymerase were undertaken. Various tissue specimens with known viral content were used as positive controls, in contrast to the standard PCR in which various DNA extracts with known viral content were used.

Fourteen (34%) of 41 breast cancer specimens gave a false-positive signal when the primers were omitted. The use of exo-zap, filling in with Klenow first, and doing two rounds of PCR, the first with no DIG-11-dUTP and the second with DIG-11-dUTP, gave the same false-positive results with these particular specimens. Consequently, we carried out a (no primer) negative control for each sample at the same time as the *in situ* screening PCR and eliminated the specimens that gave false-positive color signals.

To confirm the validity of the outcomes of *in situ* PCR analyses, DNA was extracted from the same formalin-fixed specimens, including those specimens that gave false-positive signals, and standard PCR analyses were conducted. The methods used were identical to those outlined above for standard PCR. The products were sequenced and the identity of the sequences was determined using the BLAST alignment system.

**Immunohistochemistry**

We used standard IHC techniques to assess the expression of MMTV envelope glycoprotein 52 (gp52) and Wnt1 in the archival breast cancer and mouse mammary tumor specimens. We used rabbit antibodies against MMTV *env* gp52/36 donated by Janet Butel (Interdepartmental Program in Cell and Molecular Biology, Baylor College of Medicine, Houston, TX) and were prepared as described in Slagle and colleagues (27). We used Wnt-1 (G-19) goat polyclonal antibody (dilution of 1:100; incubation time, 30 minutes) heat retrieval-DAKO Pascal system 125°C for 30 seconds with an intermediate link DAKO polyclonal rabbit anti-goat immunoglobulin/horseradish peroxidase (P0449; dilution 1:200) for 30 minutes.

For Wnt-1, the intensity of staining was assessed by eye on a 0 to 5 scale.

**Histology**

Mouse mammary tumors and human breast cancer specimens stained by H&E were examined by two independent examiners without prior knowledge of the MMTV status of the specimens. An assessment was made of the histologic similarity between the human and mouse tumor specimens. These assessments were categorized into MMTV-positive and MMTV-negative breast cancer specimens. This is an extension of our previous studies (28).

![Figure 1. MMTV-positive human DCIS breast tumor identified by *in situ* PCR and IHC. A, B, C and D are from the same human breast tumor (case 65471). A, MMTV-positive human breast cancer cell nuclei by *in situ* PCR. B, same human breast tumor specimen as in A. No PCR primer control in *in situ* PCR showing unstained nuclei. This also confirms that the DNA was not sheared and capable of self-priming. C, positive MMTV gp52 expression in proliferative areas of the same human breast cancer specimen by IHC. D, positive Wnt-1 expression by IHC, same human specimen.](#)
Nucleotide sequence comparisons
The published (Gen Bank) nucleotide sequences of selected genes from MMTV-like virus sequences identified in human breast cancer were compared with the corresponding set of sequences identified in wild mice from our own published studies of wild mice in Australia and with published exogenous and endogenous MMTV sequences from three inbred strains of mice (C57BL/6J, C3H/HeJ, and Mtv1) using both BLAST and CLUSTAL (26, 29–33).

Phylogenetic analysis
The sequences from the env region of MMTV from inbred mouse strains (C57BL/6J, C3H/HeJ, C3H, and Mtv-1), Australian wild mice, human breast cancers, and liver diseases from various geographic locations were aligned using the CLUSTAL tool in MEGA (7, 29–34). The multiple sequence alignment was used to construct a phylogenetic tree using the Neighbor Joining method (Fig. 2). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site.

Statistics
To determine if there were differences and correlations in expression of Wnt 1 between the MMTV-positive and MMTV-negative breast cancer, the Mann-Whitney and Spearman tests for nonparametric data were used (95% probability). To determine the significance of any differences between the MMTV nucleotide and protein sequences identified in human breast cancer and mouse mammary tumors, the Mann-Whitney test was used. These tests were performed using the SPSS statistical package.

Results
PCR screening for MMTV env sequences
Using standard PCR techniques, MMTV-like virus env nucleotide sequences were identified in 33 (45%) of 74 archival breast cancer specimens but were not identified in any of 29 noncancer normal breast tissue specimens. Contamination of DNA seems to be an unlikely source of the PCR signals because DNA sequencing revealed variations in the MMTV sequences detected. Nevertheless, these sequence variations were relatively minor (see later). This confirms and extends data that has previously been published (25).

Using in situ PCR, MMTV env gene sequences were identified in the nuclei of breast cancer epithelial cells in 5 (19%) of 27 breast cancer specimens (Fig. 1A) and 3 (17%) of 18 noncancer normal breast specimens. Positive (MMTV-containing mouse genomic DNA) and negative (no PCR primers; Fig. 1B) controls gave the expected outcomes. DNA was extracted from the same fixed specimens and were analyzed by standard PCR to confirm the findings based on in situ PCR. PCR products were obtained and their positive MMTV env identity was confirmed by sequencing of the PCR products.

It is of interest that 5 of the 14 specimens, which were eliminated because of "false positives" with in situ PCR, gave positive signals for MMTV env sequences when standard PCR was used. The reason for the false-positive signals is unclear but does not seem to be due to contamination of the samples. This is unfortunate because it means that the data based on in situ PCR can only be used to make estimates of the minimum prevalence of the presence of these viruses.

Table 1. Breast cancer. Correlations between the presence of MMTV env sequences and Wnt-1 expression

<table>
<thead>
<tr>
<th>Average Wnt-1 expression scale, 0–5</th>
<th>Difference in Wnt-1 expression MMTV positive and negative*</th>
<th>Correlation MMTV &amp; Wnt-1†</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMTV pos n = 10</td>
<td>4.0</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>MMTV neg n = 13</td>
<td>2.8</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>IDC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMTV pos n = 18</td>
<td>3.8</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>MMTV neg n = 18</td>
<td>2.4</td>
<td>P = 0.008</td>
</tr>
<tr>
<td>Normal breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMTV pos n = 0</td>
<td>0.0</td>
<td>P = 0.00</td>
</tr>
<tr>
<td>MMTV neg n = 27</td>
<td>0.33</td>
<td>P = 0.00</td>
</tr>
</tbody>
</table>

Abbreviations: DCIS, DCIS breast cancer; pos, positive; neg, negative; IDC, IDC breast cancer; cc, correlation coefficient.
* Mann-Whitney nonparametric U test (two tailed).
† Spearman nonparametric correlation (two tailed).

MMTV gp52 and Wnt-1 by IHC
MMTV gp52 protein levels were readily detectible in six selected MMTV-like virus–positive breast tumors and in three selected MMTV-positive mouse mammary tumors. These outcomes confirmed the detection of MMTV-like virus DNA by in situ PCR (Fig. 1A). Detection of the gp52 protein is shown in Fig. 1C in the same MMTV-like virus–positive human breast cancer specimen. The controls for both human and mouse specimens (omitted gp52 antibodies) were negative.
Wnt-1 protein levels were much higher in both MMTV-like virus–positive and MMTV-like virus–negative breast cancers than normal breast tissue controls as indicated by the intensity of staining by IHC (Table 1). There was significantly higher intensity of Wnt-1 protein expression in MMTV-positive ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) breast cancer specimens compared with MMTV-negative specimens (Table 1). Detection of Wnt1 protein by IHC is shown in Fig. 1D for the same MMTV-like virus–positive human breast cancer specimen.

Positive expression of gp52 and Wnt-1 is shown in an MMTV-positive mouse mammary tumor in the Supplementary Figure (A and B, respectively).

### Histology

MMTV-positive human IDC breast cancer specimens were significantly more likely than MMTV-negative specimens to have similar histologic (morphologic) characteristics to those from MMTV-positive mouse mammary tumors [Table 2; Fig. 2A (human) and B (mouse)]. Twelve (86%) of 14 MMTV IDC breast cancer specimens classified as being “very similar” to mouse mammary tumors were positive for MMTV compared with only 2 (14%) that were negative for MMTV. The overall correlations between IDC breast cancers, which were very similar, “similar,” and “not similar” to mouse mammary tumors and the identification of MMTV were statistically significant (correlation coefficient, 0.588; \( P = 0.000 \)).

As shown in Fig. 2, the very similar invasive breast cancer specimens all had histologic features common to medullary breast carcinomas. These features include large tracts of cells (over 75% of the tumor) with little stroma in between them, marked nuclear pleomorphism (variations in nuclear size and staining), no evidence of gland formation, and prominent infiltration of lymphocytes. These histologic features are common characteristics of MMTV-associated mouse mammary tumors.

### Phylogenetic analysis

A phylogenetic tree was constructed using the sequence data that was obtained (Fig. 3). The phylogenetic tree shows that the MMTV-like virus \( env \) gene sequences from human breast cancer, liver diseases, and MMTV from inbred and wild mice interspersed with each other and did not group separately. This confirms the very high homology between

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**Table 2. Histologic similarity between human breast cancer and mouse mammary tumors**

<table>
<thead>
<tr>
<th></th>
<th>Very similar</th>
<th>Similar</th>
<th>Not similar</th>
<th>Correlation between MMTV status and similarity of breast cancer specimens (Spearman)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DCIS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMTV pos (n = 7)</td>
<td>2 (29%)</td>
<td>3 (43%)</td>
<td>2 (29%)</td>
<td></td>
</tr>
<tr>
<td>MMTV negative (n = 8)</td>
<td>3 (38%)</td>
<td>1 (13%)</td>
<td>4 (50%)</td>
<td></td>
</tr>
<tr>
<td><strong>IDC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMTV pos (n = 29)</td>
<td>12 (41%)</td>
<td>15 (52%)</td>
<td>2 (7%)</td>
<td>( cc = 0.588 ) ( P = 0.000 )</td>
</tr>
<tr>
<td>MMTV negative (n = 28)</td>
<td>2 (7%)</td>
<td>9 (32%)</td>
<td>17 (61%)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** % rounded.

Abbreviations: \( s \), significant at 95% two tailed tests; DCIS, DCIS breast cancer (the numbers are too few to allow meaningful statistical analyses.

*Spearman nonparametric correlation (two tailed).
MMTV env viral sequences identified in wild and inbred laboratory mice, human liver diseases, and human breast cancer from the United States, Australia, Italy, and Mexico. This indicates that the same MMTV-like virus env sequences are present in mice and humans.

**Nucleotide sequence comparisons**

The sequence homology comparisons were made between segments of the main five MMTV genes (LTR, gag, pro, pol, and env) in experimental inbred and wild mice, and in human breast cancer. Homologies varied between 94% and 99% (Fig. 4). The differences in homology between human and mouse MMTV nucleotide sequences are not statistically significant (on average, \( P = 1.0 \)). As the differences in outcomes between BLAST and CLUSTAL were virtually identical, only the data based on BLAST is shown.

The homology varied between 90% and 100% for the predicted amino acid sequences of differing MMTV-like viruses.
identified in human breast tumors compared with MMTV protein sequences from C3H mouse mammary tumors.

**Discussion**

The aims of this study were to consolidate the evidence that an MMTV-like virus is involved in human breast cancer. The major findings are as follows: (a) we have confirmed the presence of MMTV-like virus *env* gene sequences (using PCR and IHC) in ~40% of human breast cancer specimens. Localization of the MMTV *env* sequences to the nuclei of breast cancer cells supports our previous report (35) and indicates integration of the provirus in human breast cancer. This is important because it indicates a true infection of the breast cancer cells by the MMTV-like virus. MMTV gp52 protein levels were readily detectable in selected MMTV-like virus–positive breast tumors and in selected MMTV-positive mouse mammary tumors. (b) Wnt-1 protein expression was significantly higher in both MMTV-positive DCIS and IDC breast cancer specimens than MMTV-negative breast cancer and normal breast specimens. (c) A significant majority of MMTV-positive IDC specimens had histologic similarities to MMTV-positive mouse mammary tumors. (d) Homology comparisons of MMTV from wild and inbred strains of mice with MMTV found in human breast cancer suggests that the MMTV-like virus *env* gene could be from the same virus in both species.

**Validity of the data**

The localization of MMTV-like virus *env* by *in situ* PCR in breast DCIS and IDC specimens in which MMTV *env* sequences had previously been identified by standard PCR confirmed previous findings, some of which involved the same specimens (35). Although the immunohistochemical-based data obtained with anti-gp52 antibodies is of interest, it has to be noted that previous studies have raised concern about the possibility of the presence of cross-reacting proteins leading to a positive signal, particularly those encoded by human endogenous retroviruses (36–39). The observation that Wnt-1 is significantly more highly expressed in MMTV-positive than MMTV-negative human breast specimens, is interesting, but has to be treated with caution because Wnt-1 is expressed in many different cancers, in which its expression may be elevated even in the absence of MMTV. Moreover, assessment of Wnt-1 expression is very subjective and the numbers in this study are small. On the other hand, the very low Wnt-1 expression in normal breast specimens was striking and obviously different from the breast cancer specimens. It is of interest that positive expression of gp52 and Wnt-1 in MMTV-positive mouse mammary tumors is very similar to their expression in MMTV-positive human breast cancer specimens.

Histologic assessments are also subjective and any similarities may be due to other causes and not necessarily related to MMTV. Again on the other hand, some MMTV-positive IDC breast cancer specimens are very similar to MMTV-positive mouse mammary tumors that at a cellular level, it is not possible to distinguish between the human and mouse tumors. In our opinion, the homology comparisons are probably valid because of the identification of nearly identical MMTV *env* sequences in breast tumors in

<table>
<thead>
<tr>
<th>Inbred mice C3H/HeJ</th>
<th>AAATCTCTCTAGAGTCCCACTGAGATTTTTCTGTGTTTTTCTCCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbred mice C3H</td>
<td>AAATCTCTCTAGAGTCCCACTGAGATTTTTCTGTGTTTTTCTCCC</td>
</tr>
<tr>
<td>Wild mice</td>
<td>AAATCTCTCTAGAGTCCCACTGAGATTTTTCTGTGTTTTTCTCCC</td>
</tr>
<tr>
<td>Inbred mice C57BL/6J</td>
<td>AAATCTCTCTAGAGTCCCACTGAGATTTTTCTGTGTTTTTCTCCC</td>
</tr>
<tr>
<td>Human breast Australia</td>
<td>AAATCTCTCTAGAGTCCCACTGAGATTTTTCTGTGTTTTTCTCCC</td>
</tr>
<tr>
<td>Human breast Mexico</td>
<td>AAATCTCTCTAGAGTCCCACTGAGATTTTTCTGTGTTTTTCTCCC</td>
</tr>
<tr>
<td>Human breast Tunisia</td>
<td>AAATCTCTCTAGAGTCCCACTGAGATTTTTCTGTGTTTTTCTCCC</td>
</tr>
<tr>
<td>Human breast U8</td>
<td>AAATCTCTCTAGAGTCCCACTGAGATTTTTCTGTGTTTTTCTCCC</td>
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</tbody>
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![Figure 4. Selected MMTV envelope gene DNA sequences from inbred and wild mice and human breast cancer. ★, identical homology between individual nucleotides in all nine sets of sequences. The overall identical homology is 90%. This indicates that it is the same MMTV *env* gene in both humans and mice.](image-url)
women from many different populations with the PCR analyses conducted in different laboratories.

The phylogenetic analyses are in accord with those of Etkind and colleagues (7) who has clearly showed that MMTV-like long terminal repeat nucleotide sequences identified in human breast tumors, human non–Hodgkin’s lymphomas, and human primary biliary cirrhosis tissues do not cluster as two distinct MMTV species. However, full-length MMTV-like virus sequences from humans have to be compared with full-length MMTV in mice before definitive conclusions can be made about the two viruses being the same, although the envelope genes of retroviruses are known to show the most sequence divergence.

**Interpretation of the data**

Our current observations in DCIS and IDC breast cancer specimens validate the previous experimental evidence that MMTV can infect, multiply, and randomly integrate into the DNA of normal human breast epithelial cells (8–10). Together, these data suggest a possible causal role for MMTV-like virus in some human breast cancers. A minority (25%) of invasive human breast tumors have very high levels of similarity to both human medullary type breast cancers and mouse mammary tumors. Therefore, it is possible that MMTV-like virus is associated with this small proportion of human breast tumors despite the consistent finding that MMTV-like virus sequences are present in ∼40% of breast tumors. In feral mice, MMTV can be identified in ∼50% of some mouse populations but aged feral mice from the same population only develop a low incidence (∼20%) of mammary tumors (40). It is possible that MMTV-like virus in humans may integrate randomly into the human breast epithelial cell genome and, despite being detectable at a high prevalence (∼40%) in human breast tumors, may only lead to promotion of oncogenes in a subset of these cases (10).

If MMTV-like viruses were causally involved in the development of some breast cancers, then it would be expected that MMTV-like virus would be present in normal breast tissue although at significantly lower frequencies than that found in breast cancer. In this study, we identified MMTV-like virus env sequences by in situ PCR in 3 (17%) of normal breast specimens originating from cosmetic surgery. We have previously reported the identification of MMTV in noncancerous liver (34). The increase in prevalence of MMTV-like virus env in breast cancer (40% in breast cancer compared with 17% normal breast tissue) also supports a possible causal role in breast cancers. We have no explanation for the identification of MMTV env gene sequences by in situ PCR in several normal breast specimens in this study, whereas MMTV env gene sequences were not identified in any normal breast specimens in our previous investigations (25) but clearly by analogy to the situation in mice, normal, nonmalignant mammary epithelial cells can express MMTV in the absence of tumorigenesis (12).

In this study, we have concluded that most DCIS breast cancer specimens are not histologically similar to mouse mammary tumors. This judgment was based on the “in situ” characteristics of DCIS, whereas most mouse mammary tumors contain invasive sheets of cells. However, it is possible that many DCIS human specimens have an association with MMTV. This is because the cancer cells, as distinct from the macroscopic appearance of DCIS, are similar to the cancer cells in many mouse mammary tumors (see Fig. 2). In addition, we have previously shown that a high proportion of human DCIS contains MMTV env sequences (25) and also Wnt-1 expression is very high in human DCIS (see Table 1).

The observation that Wnt-1 expression is significantly higher in MMTV env–positive than MMTV env–negative human breast cancers is of considerable interest. This is because the pioneering work of Nusse and Varmus (41, 42) showed that MMTV infections and subsequent integration into the genome of mouse models resulted in the activation of what is now known as Wnt-1. They also showed that Wnt-1 had an oncogenic potential. If MMTV plays a role in breast cancer in humans, as in the mouse, it may also act by preferentially integrating in the vicinity of cellular proto-oncogenes such as Wnt-1.

Mant and Cason (43) have argued that MMTV cannot have a role in human breast cancer because of the alleged histologic differences between mouse mammary tumors and human breast cancers. At low magnification MMTV-associated mouse mammary tumors do have different characteristics from human breast cancers; however, in some invasive breast cancers, the similarities can be striking in some breast cancers at a cellular level (28). Wellings (44) observed these similarities ~30 years ago.

In this study, we have not considered the possible means of transmission of MMTV. In the mouse, MMTV is most commonly transmitted through the mouse mother’s milk and other body fluids but it also can also be transmitted through the germline. It seems unlikely that MMTV is transmitted through the germline in humans, given the sensitive techniques that are available and the number of studies performed to examine this possibility. In contrast, there are various possibilities for MMTV transmission in humans. Transmission of the virus by human milk is possible despite the accepted view that there is no increase in breast cancer prevalence among women who were breast fed (45, 46). We have shown that this view is probably not correct, as the data are not based on exposure to colostrum and milk during the first weeks of life but on questions, which may be seriously misleading, because of differing interpretations by respondents about successful breast feeding (45). Importantly, we have recently shown that MMTV env sequences can be identified in ∼5% of normal mother’s milk (data not shown). There are other possible means of MMTV transmission in human populations including the ingestion of uncooked cereals, which may contain mouse fecal material (47, 48).

These findings parallel the mouse model of exogenous MMTV–associated mouse mammary tumors in wild mice. The prevalence of MMTV-associated mammary tumors in both wild field mice and wild house mice is very variable and is low in some mouse populations and high in others, and as in human breast cancer, most tumors develop late.
in life. The confirmation that MMTV sequences are present in some human breast cancers, which MMTV gp52 protein and Wnt-1 are highly expressed in some MMTV-like virus-positive breast tumors, and that a significant majority of MMTV-positive IDC breast cancer specimens had histologic similarities to MMTV-positive mouse mammary tumors, is consistent with a role of this virus in human breast cancer oncogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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Correction: Online Publication Dates for Cancer Research April 15, 2010 Articles

The following articles in the April 15, 2010 issue of Cancer Research were published with an online publication date of April 6, 2010 listed, but were actually published online on April 13, 2010:


Dudka AA, Sweet SMM, Heath JK. Signal transducers and activators of transcription-3 binding to the fibroblast growth factor receptor is activated by receptor amplification. Cancer Res 2010;70:3391–401. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3033.


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