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EPIDEMIOLOGY

No evidence of MMTV-like *env* sequences in specimens from the Australian Breast Cancer Family Study

Daniel J. Park · Melissa C. Southey · Graham G. Giles · John L. Hopper

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Abstract Numerous independent groups from a range of countries have reported a high prevalence of Mouse Mammary Tumour Virus (MMTV)-like *env* sequences in human breast cancer specimens, including a prevalence of almost 40% in Australia. MMTV-like *sag* sequences and a completely integrated provirus have also been described. Recently, it was reported that MMTV is capable of productive infection of human breast cells in vitro. Conclusive demonstration of an association between MMTV and human breast cancer has remained elusive, and negative findings from a number of independent studies have questioned the role of MMTV as an aetiological agent. We used breast cancer specimens from women in the Australian Breast Cancer Family Study (ABCFS) who were diagnosed with first primary invasive breast cancer before the age of 40 years. Specimens were selected for higher grade cancers and for diagnosis relatively soon after childbirth. We searched for MMTV-like *env* sequences in tumour-enriched DNA using a nested PCR designed to detect all MMTV variants represented in GenBank, including those reportedly detected in human breast cancers. Forty-two specimens were deemed adequate for testing based on strong *β-globin* PCR. Despite the MMTV nested PCR regimen consistently detecting five copies of control plasmid against a background of MMTV-negative human genomic DNA, no MMTV *env* sequence was detected in any of the breast cancer specimens. Our findings appear inconsistent with previous reports on Australian breast cancer specimens but consistent with a growing number of independent negative reports internationally. We recommend caution in inferring a role for MMTV or a closely related virus in human breast cancer and suggest that universally regarded alternative lines of evidence such as highly specific serology data will be required to support such an association.

Keywords Mouse Mammary Tumour Virus · Human Mammary Tumour Virus · Human MMTV-like virus · Australian Breast Cancer Family Study · Nested PCR

Introduction

Worldwide, approximately 800,000 incident cases of breast cancer are diagnosed each year [1]. For several decades, the retrovirus Mouse Mammary Tumour Virus (MMTV) or a close relative (collectively termed 'MMTV') has been suggested as a possible aetiological agent for human breast cancer. Aspects of the biology of MMTV and some recent reports suggest a strong case. MMTV was the first virus shown to cause cancer in mammals [2]. In the mouse, MMTV can be transmitted through the germline or via milk to suckling pups. Tumorigenesis occurs due to disruptive non-site-specific proviral integration in mammary epithelia during puberty and pregnancy [3]. MMTV *env* sequences have been reported at relatively high frequencies

D. J. Park · M. C. Southey
Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Parkville, VIC 3010, Australia

G. G. Giles
Cancer Epidemiology Centre, Cancer Council Victoria, 100 Drummond Street, Carlton, VIC 3053, Australia

G. G. Giles · J. L. Hopper (✉)
Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, Melbourne School of Population Health, The University of Melbourne, Level 1, 723 Swanston Street, Carlton, VIC 3053, Australia
e-mail: j.hopper@unimelb.edu.au

in unselected breast cancer specimens but at dramatically lower frequencies in healthy breast tissues from affected or unaffected women or in other cancer tissues [4–12]. Frequencies in breast cancer tissues of 20–40% have been reported for women in the USA, Italy, Mexico, Brazil, Argentina and Australia, and frequencies of 12, 10–17 and 0.8% have been reported for women in Japan, China and Vietnam, respectively. The geographical variation in the incidence of female breast cancer has been reported to correlate with the regional dominance of the mouse species *Mus domesticus*, suggesting a possible zoonotic connection [13]. MMTV *env* sequences have been reported to be especially common in higher grade breast cancers [9], breast cancers diagnosed during gestation (62%) [14] or lactation [6], and cases from multiple-case families [6, 15], as well as in male breast cancers (62%) [9]. MMTV transcripts and the MMTV *sag* gene have also been reported as common in breast cancer specimens [16, 17], and a complete 9.9 kb provirus has been reported [18]. It was recently reported that MMTV is capable of active infection of the human aneuploid mammary epithelial cell line Hs578T [19].

However, MMTV's candidature as an aetiological agent for human breast cancer is controversial [20–23]. Null findings have been reported by studies of UK, Austrian, Swedish, Japanese and Italian breast cancer specimens [24–28]. In apparent discord are reports of 13.9 or 74% *env*-positivity in Tunisian breast cancer specimens [29, 30] and 4.2 or 24% in Mexican cases [31, 32].

The implications for public health, should MMTV eventuate as a causative agent for human breast cancer, are great. We sought to validate previous reports by attempting to detect MMTV *env* sequences in a population-based sample of breast cancer specimens from the Australian Breast Cancer Family Study (ABCFS) using a specifically engineered nested PCR. Studied specimens were enriched for higher grade breast cancers and cases for which diagnosis occurred relatively soon after childbirth.

Materials and methods

ABCFS specimens

Thirty-eight 5 μm and twelve 3 μm Formalin Fixed Paraffin Embedded (FFPE) breast cancer tissue sections representing breast cancers from ABCFS participants were selected to enrich for breast cancers of relatively high histological grade and cancers from cases in which there was a relatively short time between the woman's last childbirth and her date of breast cancer diagnosis. H&E stained neighbouring sections were used to confirm the presence of breast cancer tissue [33, 34].

p203 and human genomic DNA controls

The plasmid p203 in *E. coli* was purchased from the American Type Culture Collection (ATCC). p203 harbours the relevant portion of MMTV targeted by the nested PCR described here. A small-scale plasmid preparation was performed according to ATCC instructions and using the QIAprep Spin Miniprep Kit (Qiagen, Doncaster, VIC, Australia). p203 concentration was determined using the Quant-It system (Invitrogen, Mulgrave, VIC, Australia) and Qubit instrument (Invitrogen, Mulgrave, VIC, Australia) according to the manufacturer's instructions. QIAamp Kit (Qiagen, Doncaster, VIC, Australia)-purified human genomic DNA from a healthy control individual whole blood sample was sourced and the concentration determined using the Quant-IT system with the Qubit instrument.

DNA extractions

Formalin Fixed Paraffin Embedded tissues (full face sections) were scraped from slides onto aluminium foil using scalpel blades and transferred to microfuge tubes using syringe needles. Separate foil, blades and needles were used for each specimen. DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, Doncaster, VIC, Australia) according to the manufacturer's instructions but with the following exceptions: histolene was used instead of xylene, two ethanol spin/wash steps were employed instead of one to facilitate thorough washing without losing tissue during aspiration of supernatant, no RNaseA was used, and elution was performed using 50 μl ATE buffer.

β-Globin PCR specimen adequacy check

GH20 and PC04 *β-globin* PCR primers [35] were ordered from Integrated DNA Technologies (IDT) at standard desalting grade. PCRs were performed in 30 μl reaction volumes containing 1.5 U AmpliTaq Gold (Applied Biosystems, Mulgrave, VIC, Australia), 200 μM dNTPs (Bioline, Alexandria, NSW, Australia), 100 nM PC04 and GH20 primers and 5 μl template DNA and overlaid with 25 μl molecular biology grade mineral oil (Sigma-Aldrich, Sydney, NSW, Australia) added from a fresh aliquot by Gilson pipette. PCR was performed using the following thermal parameters: 95°C for 10 min, followed by 40 cycles of 94°C for 45 s, 56°C for 45 s and 72°C for 45 s, followed by 72°C for 5 min. PCR products (268 bp) were analysed using agarose gel electrophoresis.

Nested PCR primers and primer design

ClustalW2 multiple sequence alignments of MMTV *env* were performed using GenBank-deposited sequences to

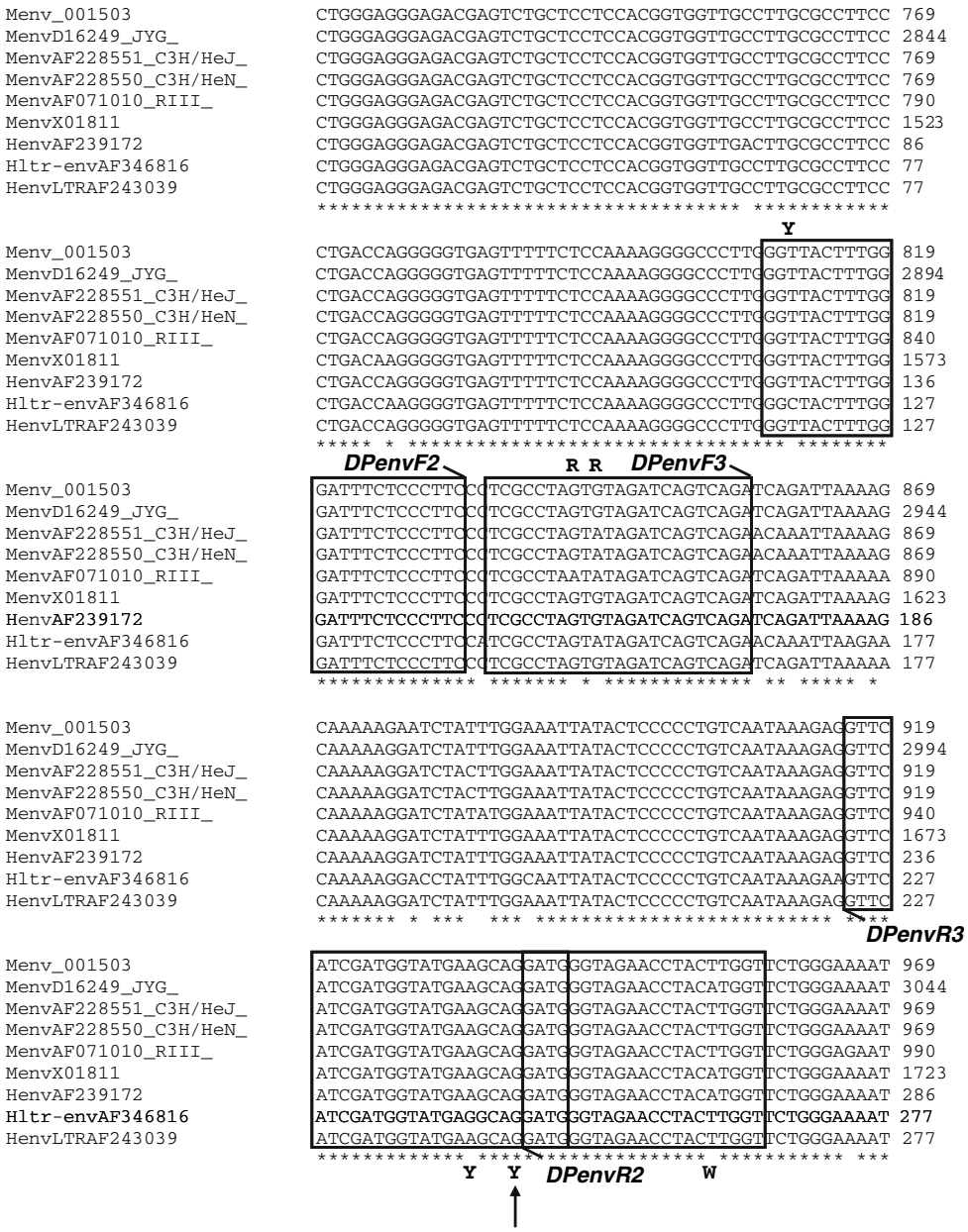

Fig. 1 ClustalW2 multiple sequence alignment of MMTV *env* within the human breast cancer-associated region [4]. The range of MMTV strains includes strains reported to be derived from human breast cancer tissue (prefixed 'H'). Database accession codes for the respective sequences are NC_001503, D16249, AF228551, AF228550, AF071010, X01811, AF239172, AF346816 and AF243039. *Boxed arrows* indicate nested PCR primer binding sites used in this study. These are annotated with the degenerate bases included in the primers (*Y*, *R* or *W*). The *vertical arrow* indicates degeneracy designed into primer DPenvR3 based on the sequences AY600606, AY600599, AY600611, AY600614, AY600604, AY600605, AY600597 and AY600608 which, together with AY600615, AY600613, AY600610 and AY600598 [9], were also aligned to aid primer design (not shown)

include a range of MMTV strains and sequences reported to be derived from human tissue specimens (see Fig. 1). Primer sites were selected with a view to yielding small amplicons (less than 200 bp), limiting the degree of mismatch across the aligned sequences, especially near the 3-prime terminus, targeting a GC content approaching 50% without excessively long GC runs, and avoiding unintended 'off target' priming from HERV-K10 (the reported most similar human endogenous retrovirus) or the human genome, excessive primer secondary structure formation and primer-dimer formation. Primer-BLAST, OligoAnalyzer 3.1 and ClustalW2 were employed to facilitate these design requirements. Remaining points of mismatch with respect to the alignment group were addressed by introducing degeneracy at corresponding primer positions. 5-prime clamp sequence was included to facilitate primer participation by all members of the degenerate primer 'pool'. Resulting first round PCR primers were (5′ to 3′, lower case indicates 5′ clamp sequence): DPenvF2: ctatagggGGYTACTTTGGGATTTCTCCCTTC and DPenvR2: ctatagggACCAWGTAGGTTCTACCCATC. Resulting second round (nested) PCR primers were (5′ to 3′): DPenvF3: ctatagggTCGCCTARTRTAGATCAGTCAGA and DPenvR3: ctatagggCATCYTGCYTCATACCATCGATGAAC. Nested PCR primers were ordered from IDT at standard desalting grade. Anticipated product sizes from PCRs employing DPenvF2 and

DPenvR2 or DPenvF3 and DPenvR3 are 166 and 123 bp, respectively. MMTV *env* sequences spanned by these PCRs are 150 and 107 bp, respectively.

Nested PCR

For assay sensitivity assessment, nested PCRs (PCR1 containing DPenvF2 and DPenvR2, followed by PCR2 containing DPenvF3 and DPenvR3) were performed using different cycle numbers (30 or 35 cycles for each PCR) or annealing temperatures (50–56°C) using decreasing copies of p203 (tested down to five copies) against a backdrop of 10 ng of MMTV-negative human genomic DNA, followed by gel electrophoretic analysis. All conditions clearly detected five copies of p203 against a backdrop of 10 ng MMTV-negative human genomic DNA. In all cases 10 ng MMTV-negative human genomic templates and water controls yielded negative results. Conditions selected to screen the breast cancer specimens were: PCR1: 30 µl reaction volumes containing 1.5 U AmpliTaq Gold (Applied Biosystems, Mulgrave, VIC, Australia), 200 µM dNTPs (Bioline, Alexandria, NSW, Australia), 100 nM DPenvF2 and DPenvR2 primers and 5 µl template DNA overlaid with 25 µl molecular biology grade mineral oil (Sigma-Aldrich, Sydney, NSW, Australia) added from a fresh aliquot by Gilson pipette. PCR1 was performed using the following thermal parameters: 95°C for 10 min, followed by 35 cycles of 94°C for 45 s, 50°C for 45 s and 72°C for 45 s, followed by 72°C for 5 min. PCR2: 30 µl reaction volumes containing 1.5 U AmpliTaq Gold (Applied Biosystems, Mulgrave, VIC, Australia), 200 µM dNTPs (Bioline, Alexandria, NSW, Australia), 100 nM DPenvF3 and DPenvR3 primers and 2 µl PCR1 product template overlaid with 25 µl molecular biology grade mineral oil (Sigma-Aldrich, Sydney, NSW, Australia) added from a fresh aliquot by Gilson pipette. PCR2 was performed using the following thermal parameters: 95°C for 10 min, followed by 35 cycles of 94°C for 45 s, 55°C for 45 s and 72°C for 45 s, followed by 72°C for 5 min. These conditions were selected as sufficiently relaxed to enable efficient specific amplification in cases of unforeseen primer-template mismatch whilst yielding 'clean' product profiles. PCR2 product was sequenced using DPenvF3 as the sequencing primer and BigDye3.1chemistry (Applied Biosystems, Mulgrave, VIC, Australia).

Agarose gel electrophoresis

2.4% Agarose TBE gels containing ethidium bromide were loaded with 5 µl PCR products alongside 250 ng of 100 bp ladder (New England Biolabs distributed by Genesearch, Arundel, QLD, Australia). Following electrophoresis, gels were photographed under UV light transillumination.

Results and discussion

Fifty ABCFS specimens were tested for 'sample adequacy' using PCR to amplify *β-globin* sequence. Strong bands of 268 bp following PCR and agarose gel electrophoresis were interpreted as being derived from adequate specimens, and the corresponding specimens were subsequently tested for MMTV *env* sequences by nested PCR. A relatively conservative approach was applied, faint products of

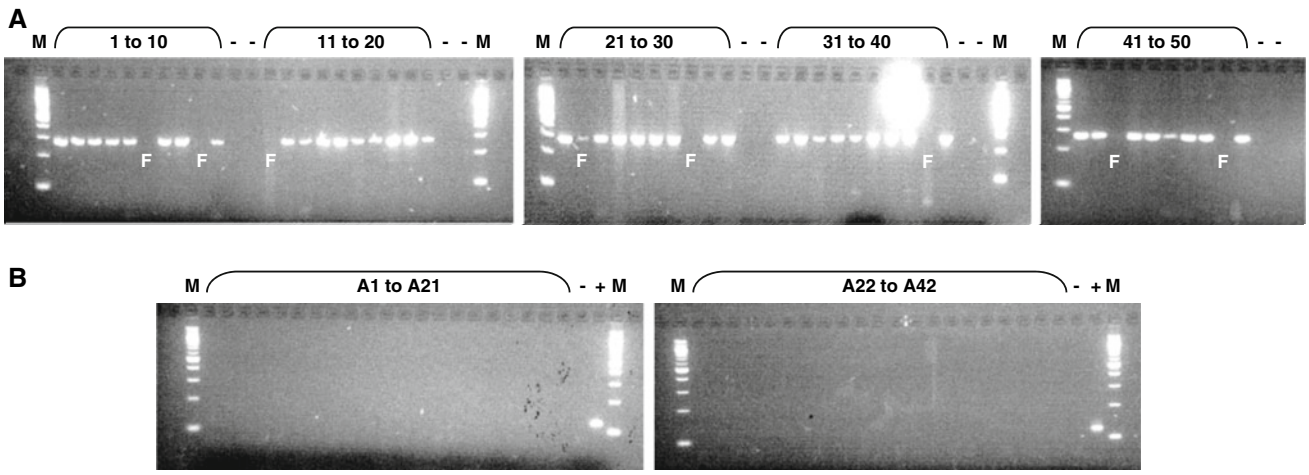

Fig. 2 2.4% Agarose TBE gels depicting the assessment of ABCFS specimen adequacy following *β-globin* PCR (**a**) and MMTV *env* sequences following nested PCR (**b**). '*M*' indicates 250 ng 100 bp ladder marker; '−' indicates a no template control; *numbers 1–50* indicate the 50 ABCFS specimens screened for specimen adequacy; '*F*' indicates a specimen interpreted as inadequate; *A1–A42* indicate the 42 adequate specimens tested for MMTV *env* sequence by nested PCR; '+' indicates a 'five copies of p203 with 10 ng MMTV-negative human genomic DNA' control

268 bp or no apparent products of 268 bp were interpreted as being derived from inadequate specimens and the corresponding specimens were not subsequently tested for MMTV *env* sequences. Of the 50 original specimens, 42 were deemed adequate (Fig. 2a), derived from thirty-four 5 μm and eight 3 μm sections.

All 42 adequate specimens represented in this study were collected from women living in the Melbourne (22/42) or Sydney (20/42) metropolitan areas that were diagnosed with invasive breast cancer before the age of 40 years. The mean age at time of diagnosis was 35.1 years (28–39 years), and 83.3% (35/42) of specimens exhibited infiltrating ductal profiles. 76.2% of the specimens (32/42) presented as histological grade 3 cancers, the remainder being grade 2 cancers. Overall, 78.6% of women (33/42) had given birth and 90.9% of specimens (30/33) were from women who had breastfed. Of the women who had given birth, the median time from the last birth to date of diagnosis was 54 months (0–244 months). Based on previously reported prevalences of MMTV *env* detection for Australian breast cancers (30–40%) [8, 9], reported increased prevalence for higher grade breast cancers [9] and reported increased prevalence for cases diagnosed during gestation (62%) [14] or lactation [6], approximately 30–50% of the 42 breast cancer specimens included in this study might have been expected to harbour MMTV *env* sequences, i.e. approximately 12–21 specimens. From the Poisson distribution, the probability of observing zero prevalence is 0.000006 when the true prevalence is 30%, and 0.05 when the true prevalence is 7%. That is, the true prevalence for Australian women is highly unlikely to be as high as previous reports, though we cannot exclude a low prevalence.

The nested PCR regimen used in this study to look for MMTV *env* sequences proved to be robust and extremely sensitive. In all experiments conducted, each using fresh dilutions of p203 to yield five plasmid copies against 10 ng MMTV-negative human genomic DNA as a parallel sensitivity control for separate PCR runs, MMTV *env* was detected as a strong 123 bp band following agarose gel electrophoresis. 'No template' controls always yielded profiles free of visible PCR product. Sequencing and BLAST analysis of the assay sensitivity control products confirmed the detection of MMTV *env*-derived sequences. None of the 42 adequate breast cancer specimens tested yielded visible MMTV *env*-derived products (Fig. 2b). Given the nature of the sample set tested and the demonstrated high sensitivity of the nested PCR regimen employed, including design features and PCR conditions to account for unforseen discrepancies between potential sample set MMTV *env* sequences and sequences included in the primer design alignment group, it seems unlikely that any of the 42 adequate ABCFS specimens tested contained MMTV *env* sequences. These findings appear inconsistent with previous reports of associations between MMTV and human breast cancer and, in particular, reports of 30–40% prevalence for unselected Australian breast cancer specimens [8, 9]. Given the risk of PCR contamination and the potential to contribute to false findings [22], we recommend caution when interpreting findings derived from highly sensitive PCR regimens which are not consistently supported by other studies.

Mouse Mammary Tumour Virus has not been found in human breast milk and is not detected in healthy tissue adjacent to cancerous tissue in humans, unlike in the mouse, and the histopathology of murine MMTV tumours is dissimilar to human breast cancers (hyperplasias vs. typically invasive ductal carcinomas) [36]. In a large study, post-menopausal women who were breastfed as infants did not have a higher risk of breast cancer compared with those who were not [37], and a separate study has reported that human breast cancer rates have increased at the same time that breastfeeding rates have decreased [38]. Pregnancy in humans appears to have a protective effect with respect to breast cancer risk, unlike MMTV-associated breast cancer in the mouse [39, 40], and serological conversion to yield antibodies cross-reactive with MMTV in human breast cancer patients remains to be demonstrated at a population level [41] despite the fact that mice produce strong antibody responses to MMTV. Further, Transferrin Receptor 1 acts as an MMTV entry receptor in the mouse but not in humans [42, 43].

The above observations together with the findings presented in this study and a growing number of null findings from independent groups internationally suggest that MMTV is unlikely to be a causative agent for female breast cancer. Alternative sources of evidence, for example, derived from universally accepted specific serological analysis will probably be required to definitively demonstrate an association between MMTV and female breast cancer. Consistent demonstration by independent groups of such an association and the detection of MMTV infection prior to the development of disease will be required to demonstrate causality [44, 45].

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Conflict of interest statement The authors declare no conflicting interests pertaining to this study.

References

1. Parkin D, Pisani M, Ferlay J (1999) Global cancer statistics. CA Cancer J Clin 49:33–64
2. Bittner JJ (1936) Some possible effects of nursing on the mammary gland tumor incidence in mice. Science 84:162
3. Ross SR (2008) MMTV infectious cycle and the contribution of virus-encoded proteins to transformation of mammary tissue. J Mammary Gland Biol Neoplasia 13:299–307
4. Wang Y, Holland JF, Bleiweiss IJ, Melana S, Liu X, Pelisson I, Cantarella A, Stellrecht K, Mani S, Pogo BG (1995) Detection of mammary tumor virus ENV gene-like sequences in human breast cancer. Cancer Res 55:5173–5179
5. Etkind P, Du J, Khan A, Pillitteri J, Wiernik PH (2000) 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
6. Pogo BGT, Melana SM, Holland JF, Mandeli JF, Polotti S, Casalini P, Menard S (1999) Sequences homologous to the mouse mammary tumor virus *env* gene in human breast cancer correlate with overexpression of laminin receptor. Clin Cancer Res 5:2108–2111
7. Melana S, Holland JF, Pogo BGT (2001) Search for mouse mammary tumor-like *env* sequences in cancer and normal breast from the same individuals. Clin Cancer Res 7:283–284
8. Ford CE, Tran D, Deng Y, Ta VT, Rawlinson WD, Lawson JS (2003) Mouse mammary tumor virus-like gene sequences in breast tumors of Australian and Vietnamese women. Clin Cancer Res 9:1118–1120
9. Ford CE, Faedo M, Crouch R, Lawson JS, Rawlinson WD (2004) Progression from normal breast pathology to breast cancer is associated with increasing prevalence of mouse mammary tumor virus-like sequences in men and women. Cancer Res 64:4755–4759
10. Holland JF, Pogo BGT (2004) Mouse mammary tumor virus-like viral infection and human breast cancer. Clin Cancer Res 10:5647–5649
11. Holland JF, Melana S, Wang Y, Fernandez-Cobo M, Jiang JD, Pogo BGT (2003) Human mammary tumor virus (HMTV) is horizontally, not vertically transmitted. Proc Am Soc Clin Oncol 22:2003 (abstr 3511)
12. Luo T, Wu XT, Zhang MM, Qian K (2006) Study of mouse mammary tumor virus-like gene sequences expressing in breast tumors of Chinese women. Sichuan Da Xue Xue Bao Yi Xue Ban 37:844–846
13. Stewart TH, Sage RD, Stewart AF, Cameron DW (2000) Breast cancer incidence highest in the ranges of one species of house mouse, *Mus domesticus*. Br J Cancer 82:446–451
14. Wang Y, Melana SM, Baker B, Bleiweiss I, Fernandez-Cobo M, Mandeli SM, Holland JF, Pogo BG (2003) High prevalence of MMTV-like *env* gene sequences in gestational breast cancer. Med Oncol 20:233–236
15. Etkind PR, Stewart AFR, Wiernik PH (2008) Mouse mammary tumor virus (MMTV)-like DNA sequences in the breast tumors of father, mother, and daughter. Infect Agents Cancer 3:2
16. Wang Y, Go V, Holland JF, Melana SM, Pogo BG (1998) Expression of mouse mammary tumor virus-like *env* gene sequences in human breast cancer. Clin Cancer Res 4:2565–2568
17. Wang Y, Jiang JD, Xu D, Li Y, Qu C, Holland JF, Pogo BGT (2004) A mouse mammary tumor virus-like long terminal repeat superantigen in human breast cancer. Cancer Res 64:4105–4111
18. Liu B, Wang Y, Melana SM, Pelisson I, Najfeld V, Holland JF, Pogo BG (2001) Identification of a proviral structure in human breast cancer. Cancer Res 61:1754–1759
19. Indik S, Gunzburg WH, Kulich P, Salmons B, Rouault F (2007) Rapid spread of mouse mammary tumor virus in cultured human breast cells. Retrovirology 4:73
20. Stewart A (2003) MMTV-related *env* sequences in human breast tumor. Int J Cancer 106:138
21. Zangen R, Sidransky D (2003) Response to 'MMTV-related *env* sequences in human breast tumors'. Int J Cancer 106:139
22. Mant C, Cason J (2004) A human murine mammary tumour virus-like agent is an unconvincing aetiological agent for human breast cancer. Rev Med Virol 14:169–177
23. Mant C, Cason J (2005) Mouse mammary tumor virus and human breast cancer. Cancer Res 65:1112–1113
24. Mant C, Gillett C, D'Arrigo C, Cason J (2004) Human murine mammary tumour virus-like agents are genetically distinct from endogenous retroviruses and are not detectable in breast cancer cell lines or biopsies. Virology 318:393–404
25. Witt A, Hartmann B, Marton E, Zeillinger R, Schreiber M, Kubista E (2003) The mouse mammary tumor virus-like *env* gene sequence is not detectable in breast cancer tissue of Austrian patients. Oncol Rep 10:1025–1029
26. Bindra A, Muradrasoli S, Kisekka R, Nordgren H, Warnberg F, Blomberg J (2007) Search for DNA of exogenous mouse mammary tumor virus-related virus in human breast cancer samples. J Gen Virol 88:1806–1809
27. Fukuoka H, Moriuchi M, Yano H, Nagayasu T, Moriuchi H (2008) No association of mouse mammary tumor virus-related retrovirus with Japanese cases of breast cancer. J Med Virol 80:1447–1451
28. Zangen R, Harden S, Cohen D, Parrella P, Sidransky D (2002) Mouse mammary tumor-like *env* gene as a molecular marker for breast cancer? Int J Cancer 102:304–307
29. Hachana M, Trimeche M, Ziadi S, Amara K, Gaddas N, Mokni M, Korbi S (2008) Prevalence and characteristics of the MMTV-like associated breast carcinomas in Tunisia. Cancer Lett 271:222–230
30. Levine PH, Pogo BGT, Klouj A, Coronel S, Woodson K, Melana SM, Mourali N, Holland JF (2004) Increasing evidence for a human breast carcinoma virus with geographic differences. Cancer 101:721–726
31. Zapata-Benavides P, Saavedra-Alonso S, Zamora-Avila D, Vargas-Rodrate C, Barrera-Rodriguez R, Salinas-Silva J, Rodriguez-Padilla C, Tamez-Guerra R, Trejo-Avila L (2007) Mouse mammary tumor virus-like gene sequences in breast cancer samples of Mexican women. Intervirology 50:402–407
32. Holland JF, Melana SM, Wang Y, Bleiweiss I, Levine P, Gombe Mbalawa C, Kalengayi M, Ndom P, Ramirez M, Cervantes G, Pogo BGT (1998) Geographic variation in proportion of breast cancers with sequences homologous to MMTV env gene. Proc Am Assoc Cancer Res 39:55
33. Dite GS, Jenkins MA, Southey MC, Hocking JS, Giles GG, McCredie MRE, Venter DJ, Hopper JL (2003) Familial risks, early-onset breast cancer, and BRCA1 and BRCA2 germline mutations. J Natl Cancer Inst 95:448–457
34. John EM, Hopper JL, Beck JC, Knight JA, Neuhausen SL, Senie RT, Ziogas A, Andrulis IL, Anton-Culver H, Boyd N, Buys SS, Daly MB, O'Malley FP, Santella RM, Southey MC, Venne VL, Venter DJ, West DW, Whittemore AS, Seminara D (2004) The Breast Cancer Family Registry: an infrastructure for cooperative multinational, interdisciplinary and translational studies of the

genetic epidemiology of breast cancer. Breast Cancer Res 6:R375–R389
35. Ip SM, Wong LC, Xu CM, Cheung ANY, Tsang PCK, Ngan HYS (2002) Detection of human papillomavirus DNA in malignant lesions from Chinese women with carcinomas of the upper genital tract. Gynecol Oncol 87:104–111
36. Callahan R, Smith GH (2000) MMTV-induced mammary tumorigenesis: gene discovery, progression to malignancy and cellular pathways. Oncogene 19:992–1001
37. Titus-Ernstoff L, Egan KM, Newcomb PA, Baron JA, Stampfer M, Greenberg ER, Cole BF, Ding J, Willett W, Trichopoulos D (1998) Exposure to breast milk in infancy and adult breast cancer risk. J Natl Cancer Inst 90:921–924
38. MacMahon B, Cole P, Brown J (1973) Etiology of human breast cancer: a review. J Natl Cancer Inst 50:21–42
39. Kampert JB, Whittemore AS, Paffenbarger RS Jr (1988) Combined effect of childbearing, menstrual events, and body size on age-specific breast cancer risk. Am J Epidemiol 128:962–979
40. Nicholl CS, Tucker HA (1965) Estimates of parenchymal, stromal, and lymph node deoxyribonucleic acid in mammary glands of C3H/Crgl-2 mice. Life Sci 4:993–1001
41. Goedert JJ, Rabkin CS, Ross SR (2006) Prevalence of serologic reactivity against four strains of mouse mammary tumor virus among US women with breast cancer. Br J Cancer 94:548–551
42. Ross SR, Schofield JJ, Farr CJ, Bucan M (2002) Mouse transferrin receptor 1 is the cell entry receptor for mouse mammary tumor virus. Proc Natl Acad Sci USA 99:12386–12390
43. Wang E, Albritton L, Ross SR (2006) Identification of the segments of the mouse transferrin receptor 1 required for mouse mammary tumor virus infection. J Biol Chem 281:10243–10249
44. Evans AS, Mueller NE (1990) Viruses and cancer. Causal associations. Ann Epidemiol 1:71–92
45. Vonka V (2000) Causality in medicine: the case of tumours and viruses. Philos Trans R Soc Lond 355:1831–1841