

Mouse Mammary Tumor-Like Virus Is Associated with p53 Nuclear Accumulation and Progesterone Receptor Positivity but not Estrogen Positivity in Human Female Breast Cancer

Margaret Faedo,^{1,2} Caroline E. Ford,²
Reena Mehta,¹ Katrina Blazek,² and
William D. Rawlinson^{1,2,3}

¹Virology Division, Department of Microbiology, South Eastern Area Laboratory Services, The Prince of Wales Hospital, Randwick;
²School of Biochemistry and Biomedical Sciences, University of New South Wales, Sydney; and ³School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia

ABSTRACT

Purpose: The purpose is to compare the presence of proteins with known associations with breast cancer—progesterone receptor (PgR), estrogen receptor, and p53, with the prevalence of mouse mammary tumor virus (MMTV)-like DNA sequences in human female breast cancers.

Experimental Design: A cohort of 128 Australian female breast cancers were screened for MMTV-like DNA sequences using PCR. The presence of PgR, estrogen receptor, and nuclear accumulation of p53 protein was assessed in the same samples using immunohistochemical staining.

Results: Nuclear accumulation of p53 was significantly more prevalent ($P = 0.05$) in archival human breast cancers containing MMTV-like DNA sequences. The presence of progesterone receptor was significantly higher in MMTV-positive than MMTV-negative breast cancers ($P = 0.01$). No correlation between estrogen receptor and MMTV-like DNA sequences was found.

Conclusions: MMTV causes breast cancer in mice, and hormones up-regulate expression of virus in mice mammary tissue. It is unknown if this is the case in human breast cancers shown to contain DNA of MMTV-like viruses. The positive association between MMTV-like DNA sequences and PgR indicates hormones and MMTV may play a role in human breast cancer. Mutations of the tumor suppressor gene *p53* are common in human breast cancer and are associated with higher grades of cancer. The association of MMTV-like DNA sequences with higher grades of cancer,

and the positive association between p53 and MMTV-like DNA sequences clearly warrant additional investigation.

INTRODUCTION

The underlying causes of most cancers are largely unknown, and infectious agents remain possible etiological factors in human cancer etiology (1, 2). The mouse mammary tumor virus (MMTV) is a nontransforming retrovirus proven to cause breast cancer in susceptible mice (3). MMTV exists as both an endogenous and exogenous virus transmitted to pups via the germ line and breast milk. The virus does not contain an oncogene but inserts near a known set of proto-oncogenes resulting in up-regulation and tumorigenesis (reviewed in Ref. 3). MMTV expression within the mammary gland is dependent on elevated levels of steroid hormones, including estrogen and progesterone that activate virus transcription from the hormone response elements located in the long terminal repeat of the provirus (4, 5).

PCR specific for MMTV in humans allowed detection of MMTV-like envelope sequences within human breast cancer specimens without amplification of closely related human endogenous retrovirus sequences (HERV-K10; Ref. 6). MMTV has been found in 40% of human breast cancers in the United States (7), in 2% of normal human breast tissue, and in our laboratory, in 40% of Australian breast tumors (8). Tumorigenesis in breast cancer involves multiple factors, and we hypothesize that MMTV and other factors may act synchronously or serially to cause breast cancer (9).

A known cofactor in breast cancer is *p53*, a tumor suppressor gene that regulates cell cycle through its role as a transcriptional factor for genes that mediate DNA damage repair, growth arrest, and apoptosis. The prevalence of *p53* mutation in breast cancer varies from 16 to 40% (10–14). In many tumors, *p53* is inactivated either directly as a result of mutations in the *p53* gene or indirectly through binding to viral proteins (reviewed in Refs. 15–17). Most mutant forms of the protein have a prolonged half-life, allowing immunohistochemical detection on tissue sections. We hypothesize the prevalence of nuclear p53 is higher in breast cancers containing MMTV-like DNA but not MMTV-negative tumors. Because viral transcription of MMTV in mice is dependent upon hormone regulation, we have investigated if hormone receptor expression is higher in human breast cancers positive for MMTV by determining estrogen receptor (ER) and progesterone receptor (PgR) status of a cohort of breast cancers from 103 Australian women.

MATERIALS AND METHODS

A total of 128 deidentified samples of formalin-fixed and paraffin-embedded tissue from 128 women at the Prince of Wales hospital (Sydney, Australia) between 1993 and 2002

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Requests for reprints: Margaret Faedo, Virology Division, Department of Microbiology, South Eastern Area Laboratory Services, The Prince of Wales Hospital, Randwick, New South Wales, 2031 Australia. Phone: 61-2-9382 9096; Fax: 61-2-9398-4275; E-mail: faedom@sesahs.nsw.gov.au.

were studied. All samples were graded, and ethics approval obtained from the appropriate Research Ethics Committee (Ref: 00/189). Samples were cut into 4- μ m thick sections using a microtome, and the blade cleaned between sections to avoid cross contamination. DNA was extracted using a modification of the method outlined (8). Differences were that paraffin was not removed before proteinase K digestion, the incubation step was conducted at a higher temperature (65°C), and the tubes centrifuged at 14,000 \times *g* to separate the solution containing DNA from the solidified paraffin.

All samples were screened first for a housekeeping gene (glyceraldehyde-3-phosphate dehydrogenase) and, if positive, were then tested for MMTV-like envelope sequences using semi-nested PCR. The first round of PCR with outer primers IX and 2NR amplified a 356-bp product (6). The second round used 10 μ l of the first round product as template, with the primer pair MMTV5F-GGTATGAAGCAGGATGGGTAGA and 2NR used to amplify a 190-bp inner product (6). All PCRs were performed in a 50- μ l mixture containing buffer (10 \times), MgCl₂ (2.5 mM), deoxynucleoside triphosphates (0.25 mM), 10 pmol of each primer, and 1 unit Taq polymerase (Promega, Madison, WI).

Immunohistochemistry was conducted with standard techniques on a DAKO autostainer, using DAKO secondary biotinylated polylinker antibody and horseradish peroxidase-streptavidin (DAKO), followed by color development using diaminobenzidine solution. The primary antibodies used were as follows: p53 (D07, 1:200; MCO); ER (M7047, 1:150; DAKO); and PgR (MU-328-UC, 1:100; Biogenex).

ER, PgR, and p53 expression of tumors were scored in terms of the proportion of invasive nuclei staining (0, <10%; 1, 10–25%; 2, 26–50%; 3, >50%) and the average staining intensity (1, weak; 2, intermediate; 3, Strong). The score for intensity was added to the score for proportion, and scores of ≤ 5 were considered positive.

All statistical comparisons were performed in SPSS for Windows (student version 6.1.3). The two groups (MMTV-positive and MMTV-negative tissue) were compared in two categories (firstly p53 nuclear accumulation and no p53 nuclear accumulation; then ER positive and ER negative; and finally PgR positive and PgR negative) using χ^2 test.

RESULTS

The 128 female breast cancer samples used in this study were all infiltrating ductal carcinomas, including 37 infiltrating ductal carcinomas grade I, 44 grade II, and 47 grade III carcinomas, all with similar prevalence of MMTV-like DNA sequences (Table 1).

Table 1 Prevalence of mouse mammary tumor virus (MMTV)-like DNA sequences in breast cancer samples, infiltrating ductal carcinoma (IDC; grades I–III)

Pathology	MMTV positive	MMTV negative
IDC III	20	27
IDC II	18	26
IDC I	12	25
Total	50	78

Table 2 Immunohistochemical staining results for breast cancer samples, infiltrating ductal carcinoma (IDC, grades I–III)

	MMTV-positive	MMTV-negative	<i>P</i>
p53 ^a positive	15	12	0.05
p53 ^a negative	35	66	
ER ^b positive	10	19	0.57
ER ^b negative	40	59	
PgR ^c positive	18	13	0.01
PgR ^c negative	32	65	

^a Nuclear accumulation of p53 protein.

^b Estrogen receptor positive staining.

^c Progesterone receptor positive staining.

Immunohistochemistry was used to assess nuclear accumulation of p53, presence of ER and presence of PgR (Table 2). Using χ^2 test nuclear accumulation of p53 was significantly higher in MMTV-positive breast cancers (30%) compared with MMTV-negative (15%) breast cancers ($P = 0.05$).

The proportion of ER-positive samples did not vary significantly ($P = 0.57$) between MMTV-like envelope positive and negative samples. However, there was a significant difference in the presence of PgR in MMTV-positive (36%) and compared with MMTV-negative (17%) samples ($P = 0.01$).

The proportion of samples copositive for ER and PgR did not vary significantly between MMTV-like positive and negative samples. The proportions were: ER⁺PgR⁺ 11% (14 of 128), ER⁺PgR⁻ 12% (15 of 128), ER⁻PgR⁺ 13% (17 of 128), and ER⁻PgR⁻ 64% (82 of 128).

DISCUSSION

The prevalence of MMTV-like DNA sequences found in this breast cancer cohort (50 of 128 or 40%) compares to previous studies from this laboratory (42.2%; Ref. 8) and others (38.5 and 37%; Refs. 6 and 18, respectively). Interestingly, a correlation between p53 nuclear accumulation and the presence of MMTV-like DNA sequences in breast cancer has been shown. Wild-type p53 is a tumor suppressor, but some mutant forms of the protein are cancer promoting. Cells without wild-type p53 function are genetically less stable because division can occur without repair of the accumulating damage. In this study, immunohistochemistry was used to detect p53 nuclear accumulation. The mutant form of *p53* has a prolonged half-life, and this is the major basis of detection of p53 abnormalities on tissue sections using immunohistochemistry. It should be noted that some mutations in *p53* such as splice site mutations and premature stop codons may give rise to no protein expression, hence no staining would be observed (11). Conversely, in some circumstances, p53 can accumulate in the absence of detectable gene mutations (11), but this is uncommon.

In summary, correlation between MMTV and p53 may be a result of synergy, resulting in more cellular dysregulation and more severe cancers or simply an epiphenomenon. A possible explanation is that if a cell where MMTV DNA had been inserted near a proto-oncogene were to lose p53 wild-type function, the resultant steps of tumorigenesis resulting from up-regulation of proto-oncogenes could continue unchecked. Conversely, cells that have lost p53 wild-type function may be more likely to permit integration of foreign proviruses.

We showed no correlation between the presence of MMTV-like DNA sequences and the presence of ER. However, a significantly higher number of PgR-positive samples were found in MMTV-positive compared with MMTV-negative breast cancers. Huang *et al.* (19) investigated the proportion of ER and PgR in a Carolina-based cohort. The rates of ER⁺PgR⁻ (10%) and ER⁻PgR⁺ (8%) were comparable with our results. However, the proportion of ER⁺PgR⁺ (49%) and ER⁻PgR⁻ (33%) varied from our study, 11 and 64%, respectively.

A previous study compared the presence of p53 and PgR, along with c-erbB2, bcl-2, PdR, cathepsin D, and laminin receptor to the presence of MMTV (20). They found the number of MMTV envelope-positive tumors increased with the number of cells expressing the laminin receptor only. This conflicts with the results presented here, which shows a significant difference in both p53 nuclear accumulation and PgR positivity in MMTV-positive breast cancers compared with MMTV-negative breast cancers. The study reported here was comprised of a larger number of samples (128 in comparison to 26 in the previous study), which may explain the difference noted between the two studies. We have yet to determine the level of laminin receptor expression in this cohort.

Hormones play an important role in expression of the MMTV in the mammary tissue of mice, and MMTV is associated highly with at least one p53 mutation in mice (21). It is unknown if a similar situation exists in human breast cancer, and the results presented here suggest accumulation of p53 and expression of PgR are associated with MMTV prevalence. This furthers the argument for MMTV contributing to human breast cancer but does not prove causality. It does indicate possible cellular mechanisms by which tumorigenesis may arise, and the role of these and other hormones in MMTV-like expression is now being additionally clarified.

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