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ORIGINAL RESEARCH

Increased Detection of Breast Cancer Virus Sequences in Inflammatory Breast Cancer

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Abstract: Earlier studies have suggested an association between breast cancer aggressiveness and the presence of viral sequences resembling mouse mammary tumor virus (MMTV). This study was to determine if inflammatory breast cancer (IBC) in the United States had a higher proportion of cases with these viral sequences than non-IBC patients and if specific risk factors for the sequences could be identified. Biospecimens from 67 patients in the North American IBC Registry were selected for sequencing of MMTV env-like sequences. The presence or absence of the viral sequences was compared to progression free survival (PFS), risk factors including exogenous hormones, and tumor markers. Of the 67 cases, 44 were positive for viral sequences (VSP), 17 were negative (VSN) and six were excluded from analysis because of insufficient DNA to perform replicates. The 72% of VSP cases was significantly more than the 40% in non-IBC U.S. breast cancer patients ($p < 0.0001$). Non-significant trends suggested that VSP patients were more likely to be HER-2 neu positive and ER negative, have a stronger exposure to exogenous hormones, and have a shorter PFS than VSN patients. MMTV-related sequences appear to be related to the aggressiveness of breast cancer with a higher incidence in North American IBC than in non-IBC breast cancer.

Keywords: breast cancer virus, inflammatory breast cancer

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Introduction

As evidence for a human breast cancer virus continues to accumulate,[1–9] understanding the risk factors for harboring these sequences has become increasingly important. Recently we suggested that one such risk factor could be tumor aggressiveness[10] based on the high frequency in Tunisian breast cancer patients where aggressive tumors are more common[11] and also the apparent relationship between the percentage of positive breast cancer cases and the degree of aggressiveness, the percentage of cases with grade III tumors being significantly higher than those with grade I tumors.[12] The development of an Inflammatory Breast Cancer Registry (IBCR) with the collection of biospecimens[13] provided an opportunity to compare the presence or absence of these sequences with clinical and epidemiologic data collected systematically.

Methods

Slides from paraffin blocks of tumor tissue obtained from 67 patients in the IBC Registry were utilized in this study. The IBC Registry uses medical records as well as patient interviews to document the rapid appearance of clinical signs, such as redness, warmth and edema, and the pathologic finding of dermal lymphatic invasion. The paraffin blocks used in this study were either diagnostic pre-treatment tissue or mastectomy samples which were more abundant and provided more slides for evaluation. The cases were chosen on the basis of the number of slides available for study. The 564 non-IBC controls were a summation of U.S. breast cancer patients studied in the same laboratory using the same methodology as used for the IBC patients. The non-IBC cases were an unselected group of breast cancer patients followed in a variety of medical centers by numerous physicians and the assays were performed without the availability of clinical data. Information regarding exogenous hormones was derived from a questionnaire which was given orally to the patients when first entered into the Registry. Hormone receptor and Her2-neu data were collected from the pathology reports in the IBC patients' medical records. Patient follow-up was obtained at intervals of no greater than 12 months and progression free-survival was considered as the time between initiation of treatment and the recurrence of tumor documented by the patient's oncologist through clinical evaluation or imaging studies.

All slides were tested by one of us (H.R.) in a blinded fashion during a period where non-inflammatory as well as inflammatory breast cases were being tested. No clinical information was made available to the testing laboratory. To amplify the 250-bp of the MMTV-like env gene, DNA was extracted from the paraffin-embedded tissue sections and tested for DNA quality by amplification of the β-globin gene by polymerase chain reaction.[5] Primers 2N and 3N were used to amplify the 250-bp sequence.[3] Each sample was tested at least two times. Hybridization with a labeled internal probe (primer 2a) to identify the amplicon was performed as previously described.[3] Sequences were determined at the Mount Sinai Core Facilities. Two of them (EF 495355 and EF 495356) were communicated to the Gene Bank. The sequences were homologous to the env gene of MMTV and to published sequences from sporadic breast cancers.

Bivariable relationships between MMTV expression and various characteristics were compared using Fisher's exact test or chi-square analysis as appropriate. Multiple logistic regression was used to model the relationship between MMTV expression and the collection of characteristics to produce odds ratios and 95% confidence intervals adjusted for all variables in the model. Progression free survival (PFS) comparisons between MMTV env-positive and negative cases were tested for statistical significance using Kaplan-Meier survival methods with the log-rank chi-square.

Results

The incidence of VSP North American IBC cases (44/61 = 72%) is significantly higher than those in U.S. breast cancer cases tested in the same laboratory (226/564 = 40%) ($p < 0.0001$, chi square = 23.1).

The association of viral sequence positivity and tumor aggressiveness had its corollary in cell surface markers (Table 1). VSN patients were less likely to be ER negative (8/17 = 47%) than VSP patents (24/44 = 55%), but the differences were not significant (odds ratio = 0.74; 95% CI: 0.24, 2.28). HER2-neu receptors were also present more often (19/41 = 46%) in VSP than in VSN patients (6/16 = 38%) (odds ratio = 1.44, 95% CI: 0.44, 4.70).

The data regarding risk factors for developing VSP malignancies suggested an association with exogenous hormones (Table 1). Oral contraceptives were

Table 1. Risk factors associated with viral influence.

Risk factor		Positive viral sequence	Negative viral sequence	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)+
Oral contraceptive use					
(n = 59)*	Yes	35	11	1.99 (0.54–7.35)	2.29 (0.55, 9.51)
	No	8	5		
Hormone replacement therapy					
(n = 59)*	Yes	16	4	1.78 (0.49, 6.46)	1.37 (0.35, 5.37)
	No	27	12		
Estrogen receptor status					
(n = 61)**	Positive	20	9	0.74 (0.24, 2.28)	0.76 (0.21, 2.79)
	Negative	24	8		
Her-2 neu status					
(n = 57)***	Positive	19	6	1.44 (0.44, 4.70)	1.22 (0.32, 4.65)
	Negative	22	10		

*Not included in the analysis were 2 patients with missing risk factor data.
**Borderline positives for the receptors are included as positive (one who was viral sequence positive).
***Borderline positives for the receptors are included as positive (1 who was viral sequence positive; and 1 who was viral sequence negative), and 4 patients with no HER-2 Neu information were not included.
+Adjusted for all other variables in the table.

used more often in VSP patients (35/43 = 81%) than VSN patients (11/16 = 69%) (odds ratio = 1.99, 95% CI: 0.54, 7.35) and hormone replacement therapy was used more often by VSP patients (16/43 = 37%) as compared to VSN patients (4/16 = 25%) (odds ratio = 1.78, 95% CI: 0.49, 6.46). However, none of these differences were significant in a bivariable analysis. In addition adjusting for other characteristics in the model did not produce substantial changes in the odds ratios or significance.

Another link to cancer aggressiveness is suggested by VSP patients having a shorter median PFS (45.8 months) than VSN patients (61.4 months) but these differences were not statistically significant (chi-square = 0.36; p = 0.55).

Discussion

Since 1984[1] an increasing number of laboratories utilizing different *in situ* techniques have documented the presence of MMTV-associated antigens and viral sequences in human breast cancer tissues and not in normal tissues from the same patient. Furthermore, the localization of these specifically in the tumor cells and not in adjacent non-malignant cells provides one of the most important criteria indicating an etiologic relationship between the proposed virus and the tumor. This study was undertaken because of an earlier epidemiologic report[10] suggesting a geographical correlation between breast cancer aggressiveness and the presence of viral markers, the highest percentage of VSP patients in these studies being found in Tunisian patients reported to have an incidence of IBC in approximately 50% of their breast cancer patients.[11]

In this study, the major finding is the indication that MMTV-like related sequences correlate with tumor aggressiveness since IBC is associated with significantly higher positivity (71%) vs. non-IBC (40%). The relationship to tumor aggressiveness is supported by the finding of a progressive increase in the percentage of MMTV-related sequences with higher tumor grade and the independent association of these sequences with the laminin receptor,[14] a marker of poor prognosis. A possible role for hormone stimulation is suggested by the higher percentage of MMTV-related sequences in gestational breast cancer, which is associated with both increased hormonal

stimulation and a poorer prognosis,[15] and the finding of MMTV-related sequences in male gynecomastia.[16] Several other relevant observations include the correlation of exogenous hormone intake (oral contraceptives and hormone replacement therapy) and increased tumor grade,[17] the correlation with obesity which carries increased hormone levels and pre-menopausal inflammatory breast cancer,[18] and the association of IBC with prolonged breast feeding.[19] Of interest is the observation that hormonal stimulation enhances the replication of MMTV in mice,[20,21] known to increase the chance of integration of MMTV leading to the activation of a cellular proto-oncogene.[22]

Whether or not these MMTV-like sequences, which now have been studied in apparent viral particles from cultured breast cancer cells[9] are of etiologic significance, a marker of tumor aggressiveness, or both, considerable speculation has revolved around the explanation of the marked geographic differences in humans.[10] One hypothesis which fits well with both human and animal data is that the geographic differences in humans follows the documented patterns in wild mice, which have colonies of highly infected mice with high rates of breast cancer living separately but not too distant from colonies of less infected mice with lower breast cancer incidence.[23] The origin of the rise of these disparate patterns in mice are not known. There are numerous examples of virus induced animal malignancies that have provided useful models for human malignancy. Examples include the woodchuck hepatitis virus, which causes liver cancer in the host animals and provides a useful model for hepatitis B virus-induced human liver cancer, and feline leukemia and immunodeficiency viruses which are useful models for human HTLV-I adult T-cell leukemia/lymphoma and acquired immunodeficiency syndrome (AIDS).[24] The data we are now presenting suggests that murine breast cancer follows in this mode. In the mouse, there is no question that viral transmission is primarily from mother to child through breast feeding[25] and this accounts for the tight regional differences between groups of wild mice in California as noted above. It is possible that the proposed human breast cancer virus also is transmitted through breast feeding, as is HTLV-I in which breast feeding is a major form of viral transmission.[26]

A second hypothesis reported in the literature is that in contrast to human-to-human transmission, the proposed virus is actually transmitted from mice to humans,[27] this zoonotic transmission hypothesis getting some support from the finding that MMTV infects human cells.[28] Although the precise role of a virus in the pathogenesis of human breast cancer is yet undefined, progress in the laboratory combined with field efforts will hopefully resolve these questions in the not too distant future.

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Disclosures

The authors report no conflicts of Interest.

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