No Evidence of Mammary Tumor Virus *env* Gene-Like Sequences among Iranian Women with Breast Cancer

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Key Words

Mouse mammary tumor virus · Human murine mammary
tumor virus-like virus · Breast cancer

Abstract

Objective: The mouse mammary tumor virus (MMTV) is the well-established etiological agent of mammary tumors in mice. A series of studies have implicated that a human murine mammary tumor virus-like virus occurs in human breast cancer, but it is unclear whether it has any causal role. **Methods:** The aim of the present study was to investigate the presence of MMTV *env* gene-like sequences in a group of Iranian women with or without breast cancer. A total of 65 breast cancer and 65 noncancerous breast specimens from the Department of Pathology of Tabriz University in East Azerbaijan, Iran, were analyzed by nested PCR. **Results:** All breast cancer and benign breast samples were negative for MMTV *env* gene-like DNA. **Conclusion:** These results indicate that the MMTV *env* gene-like virus may not play a significant role in the etiology of breast cancer among Iranian women.

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Introduction

Breast cancer is the most frequent cancer in women worldwide. Epidemiological studies have suggested a number of risk factors, including family history, genetic background, environmental factors, high-fat diet, nulliparity, early age of menarche, late age of menopause, and infectious agents, including viruses [1, 2].

Viral etiology has been suspected but not yet proven for human breast cancer. The discovery by Bittner [3] in 1943 showed that mouse mammary tumor virus (MMTV) caused mammary cancers in mice. Consequently, MMTV has been considered as a potential etiological agent associated with breast cancer in humans. This assumption was supported by the proof that some investigators have found similar viral sequences, MMTV-like gene sequences (human murine mammary tumor virus-like virus), in human breast cancer [4–8]. Although there is substantial evidence that the MMTV-like virus may play a role in human breast cancer, the cause-effect theory is yet to be proven.

Fig. 1. Agarose gel electrophoresis of human murine mammary tumor virus-like virus analyzed by nested PCR of breast cancer samples (1–5). Molecular weight: GeneRuler™ 100-bp DNA ladder; positive control: MCF-7 cell line.

Table 1. General characteristics of the study population

	Cases (n = 65)	Controls (n = 65)	p value
Age			
<40	20 (30.8)	39 (45.4)	p < 0.05
40–50	21 (32.3)	19 (29.2)	
>50	24 (36.9)	7 (10.8)	
HMLTV DNA			
Positive	0 (0.0)	0 (0.0)	
Negative	65 (100)	65 (100)	
Histopathological types			
IDCI	6 (9.2)		
IDCII	36 (55.5)		
IDCIII	8 (12.3)		
ILCI	3 (4.7)		
ILCII	8 (12.3)		
Phyllodes tumor	1 (1.5)		
Metastatic tumor	1 (1.5)		
Mucinous carcinoma	1 (1.5)		
Ductal carcinoma	1 (1.5)		
Fibrocystic lesion		53 (81.6)	
Fibroadenoma		6 (9.2)	
Lipoma		1 (1.5)	
Fat necrosis		1 (1.5)	
Epidermal cystic lesion		2 (3.2)	
Lymph node		1 (1.5)	
Ruptured epidermal cystic lesion		1 (1.5)	

Values are presented as numbers (with percentages). HMLTV = Human murine mammary tumor virus-like virus; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma.

Breast cancer is the most frequent malignancy among Iranian women [9]. However, the epidemiological aspects of breast cancer among Iranian patients are uncertain [10]. Due to the potential importance of an association between MMTV and some cases of human breast cancer, we screened a group of Iranian women with or without breast cancer for the presence of MMTV-like sequences in breast tissues.

Materials and Methods

A total of 65 formalin-fixed paraffin-embedded breast cancer tissue blocks and 65 noncancerous breast specimens were obtained from the Pathology Department of Tabriz University in East Azerbaijan in Iran. All samples were cut and collected in sterile tubes. To avoid possible cross-contamination between specimens, special care was taken by using disposable blades and changing gloves between cutting the blocks. Deparaffinization was performed according to protocols mentioned in previous studies [11]. DNA from all samples was extracted by a QIAamp DNA FFPE tissue kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). For each sample, PCR amplification with specific primers targeting a 268-bp fragment of the β -globin gene was carried out as an internal control to assess the quality of extracted DNA [12]. To identify MMTV-like sequences in breast cancer and control groups, nested PCR was carried out with primer pairs (targeting an amplicon size of 660 and 250 bp) and protocols, as mentioned in previous studies [13].

A reaction mixture containing genomic DNA, extracted from the MCF-7 human breast cancer cell line that expresses MMTV *env* gene-like sequences, was applied as a positive control. The amplified products were visualized on a 2% agarose gel (fig. 1).

Results

The characteristics of the study subjects including age and histopathological types in the case and control groups are shown in table 1. In the case and control groups, respectively, 63.1 and 74.6% of specimens were less than 50 years old. The most frequent histological type of breast cancer (55.4%) was invasive ductal carcinoma II. In the control group, the most prevalent lesion was fibrocystic (81.5%).

Tissue sections obtained from 65 breast cancer paraffin-embedded blocks and 65 noncancerous breast specimens were available for analysis of MMTV-like sequences by nested PCR. DNA extracted from tissue samples was positive for the β -globin gene in all specimens, indicating that the quality and quantity of DNA were satisfactory.

In this study, all breast cancer and benign breast samples, as well as the negative controls, were all negative for MMTV *env* gene-like DNA.

Discussion

Breast cancer affects Iranian women at least one decade younger than their counterparts in developed countries [9]. There are many published studies about breast cancer in Iran, but the epidemiological aspects of Iranian breast cancer are uncertain.

The search for a viral cause for human breast cancer has generated considerable controversy [14, 15]. MMTV has been implicated in causing mammary carcinoma in mice [3].

The contribution of MMTV in the pathogenesis of human breast cancer has long been assumed but has never been confirmed. To investigate whether MMTV-like sequences are present in breast cancer tissues in the Iranian population, 65 breast cancer and 65 benign breast samples were analyzed in this study. Our results indicate that DNA sequences that are homologous to MMTV *env* gene-like sequences were absent in all of the specimens.

Despite using a sensitive method, we did not detect MMTV-like sequences in human breast cancer tissue. The lack of MMTV-like sequences in our samples indicates that the concentration of these sequences is nil or very low in breast cancer tissue. Also, two other studies from Iran, in Tehran and Shiraz, have not found MMTV-like sequences in breast cancer specimens [16, 17].

Although it has been reported that 32–74% of human breast cancer specimens from the USA, Italy, Australia, and Tunisia contained gene sequences homologous to the MMTV *env* gene [18–21], it has not been detected in breast cancer specimens from Sweden, Austria, England, Japan, Vietnam, and Mexico [14, 20, 22–25]. Also, 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. These data have revealed that the detection of the MMTV-like sequence is diverse in different geographic regions [26]. The differences in the prevalence of MMTV-like gene sequences among populations might be associated

with host factors and the geographic distribution of different mouse species, as exogenous MMTV is detected in up to 50% of *Mus musculus domesticus* from Western countries [19, 27, 28].

If MMTV-like viruses were causal agents in the development of some breast cancers, it would be expected that MMTV-like sequences would be found in normal breast tissue in lower proportions than those found in breast cancer. In most studies, however, MMTV-like sequences were absent or rarely detected in normal human breast tissue from all populations. Also, Mant et al. [14] have argued that MMTV cannot play a role in human breast cancer because of the alleged histological differences between mouse mammary tumors and human breast cancers. Thus, the potential role of the MMTV-like virus in human breast cancer remains controversial.

This study does not support the involvement of MMTV-like sequences in Iranian women with breast cancer. Our findings imply that geographic and ethnic variations might play a significant role in MMTV-like virus infection and its role in breast cancer development. However, the role of MMTV-like sequences cannot be completely ruled out. To confirm the absence of MMTV-like sequences in breast cancer specimens, the results need to be verified by further studies with a larger sample size in different parts of Iran.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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