The Biology Behind

Mouse Mammary Tumor Virus-Like Viral Infection and Human Breast Cancer

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In the last half of the 20th century the possibility that human breast cancer might be intimately associated with onco-genic viruses was often suggested. This followed naturally on Bittner's demonstration (1) that a virus caused breast cancer in mice. Convincing data, however, eluded workers before the molecular biological revolution. Robust evidence has now been presented in a series of papers demonstrating that a sequence highly homologous to a unique 660-bp segment of the mouse mammary tumor virus (MMTV) envelope gene is present in 38% of American women's breast cancer (2, 3). The sequences were found to be expressed in most of the positive tumors (4). Subsequent polymerase chain reaction amplifications have demonstrated other gene segments homologous to their MMTV counterpart, but not to the endogenous retrovirus K10 most similar to MMTV (5). A 2.7-kb sequence of env and long terminal repeat (LTR) genes has been visualized by fluorescent in situ hybridization inserted into several chromosomes (ref. 6; Fig. 1). This too mimics MMTV, which lacks an oncogene and evokes neoplasia by its impact on genes in the near vicinity of its insertion site. Indeed, we have detected the entire viral genome from fresh breast cancers (6) and from particles isolated from their primary cultures.1 The virus contains hormone-responsive elements, which may account for the virulence of breast cancer in pregnancy and lactation; 61% of breast cancers developing during pregnancy and lactation contain the envelope sequence (7). The human isolates also contain superantigen sequences, which are functional and may contribute to pathogenesis (8). We have visualized budding virus from infective breast cancer membranes and presented preliminary data that the virus can be transmitted in vitro (9). Because of its human origin and some pervasive differences from MMTV in its LTRs, we refer to the agent as human mammary tumor virus (HMTV); (10). The viral sequences are found in breast cancer but not in normal breast tissue, thus precluding their germ-line transmission and establishing that horizontal transmission has taken place (11).

Our original report of a MMTV-like segment of the env gene in human breast cancer lead Stewart et al. (12) to a provocative possible explanation of the well-known difference of breast cancer incidence in Western and Eastern Europe. West of a narrow north-south swath through central Europe, the exclusive mouse species is Mus domesticus, the common house mouse with abundant MMTV in its genome and a high incidence of breast cancer. East of the swath, Mus musculus and Mus castaneus are the principal indigenous species, with a low content of MMTV. Given the fact that mice are co-inhabitants of nearly every human community, this striking correlation between breast cancer incidence and murine viral pools in the presence of a horizontally acquired viral infection in humans suggested trans-species infection. Inuit women, in circumpolar regions where mice do not exist, have a low incidence of breast cancer (12).

The incidence data prompted us to examine the env positivity in breast cancers in several countries west of the swath, or those colonized by Western European powers. Italy, the United States, Mexico, Brazil, and Argentina all have 30% to 40% positivity, and Tunisian breast cancer specimens are 74% positive (13). East of the swath, in China and Japan, where breast cancer incidence is known to be low, the positivity is 10% and 12%, respectively (14). Vietnamese breast cancer has been reported to be 0.8% positive (15).

The fundamental observation of MMTV-like env sequences in breast cancer has been confirmed by Etkind et al. (16), Ford et al. (15), and Levine et al. (13) and by another laboratory known to us that has not yet published its results. Other workers using different methods have not reproduced our results, but none has then reported a faithful replication of our exact methodology (17–19). A recent report using in situ polymerase chain reaction localized the product and its expression by reverse transcription-polymerase chain reaction to epithelial cells, whereas stromal cells were negative (20).

In the original paper by Etkind et al. (16), two patients with breast cancer and coexistent lymphoma were studied, and env gene sequences were found in both tumors. Elsewhere in this issue, an elaboration of their important observation is published indicating that 6 of 12 patients with both breast cancer and non-Hodgkin's lymphoma had viral sequences in each (21). This extends prior reports that non-Hodgkin's lymphoma is more common in patients with breast cancer than expected if the diseases were randomly distributed (22) and offers a possible explanation. It is well established that MMTV can evoke lymphomas in mice. Etkind et al. (21) sequenced the env sequences from the six breast cancers and six lymphomas and found that each contained a single nucleotide mutation compared with the env sequence from C3H MMTV. Only the breast cancers each contained one clone of unmutated sequence (21). This observation might represent two independent infections (the mutant virus was previously seen in a male with non-Hodgkin's lymphoma who did not have breast cancer) or high mutability of this particular strain of HMTV with selective parasitism of lymphoid...
cells only with the mutant variants. The phylogenetic analysis indicates that there are two different viruses, however. Multiple infections have also been reported in mice.

Given an association of MMTV-like viral infection and breast cancer, to posit causation it is necessary to show that the viral infection occurred first. Many prior workers have studied antibody response to MMTV in the sera of patients with breast cancer (23). An early case report details a female laboratory worker repeatedly tested because she was working with mouse breast cancer. After four negative results, her antibody titer became positive 4 months after the last determination. Nine months later, she developed a breast mass, and 1 month later, she developed an axillary mass; both subsequently proved to be cancer (24). A recent letter by Ford et al. (25) reports a control subject with env-positive benign breast tissue who subsequently developed breast cancer. Both examples support a causal association. We have demonstrated antibody response to a synthetic peptide from the env protein in breast cancer patients, but not in healthy controls. We are now engaged in studies of sera collected before the discovery of breast cancer.

The etiology of most cancers is multifactorial. Breast cancer is known to be influenced by gender and age and by environmental factors (e.g., radiation, hormones, and lifestyle). Taken together, the presence of the MMTV-like env gene in a large portion of breast cancers (2), which has been confirmed in many laboratories (13, 15, 16); the presence of the entire virus in breast cancers (6); reproduction of the virus from breast cancer cells in vitro (9); the demonstrated infectivity of env-positive breast cancer cells in vitro for normal breast cells (9); and variable penetrance in diverse populations (13, 14) present a plausible basis for considering causality. The discovery of an antibody response that indicated infection occurred before breast cancer became apparent should add to the mounting evidence that a major proportion of human breast cancer is horizontally acquired. Further research will define whether this is a new MMTV virus each time in a trans-species infection, or whether HMTV was derived from MMTV eons ago and is passed horizontally among humans. It is of note that the same virus has recently been isolated from biliary epithelium of patients with primary biliary cirrhosis and that it can infect normal biliary cells in vitro (26).

The tools of molecular biology similar to those we (6) and Etkind et al. (21) have used are appropriate to launch studies in other candidate neoplasms. Fowl, murine, feline, bovine, ape, and new world monkey leukemias are viral in origin (27). Much epidemiologic evidence suggests a possible viral role in the etiology of some human leukemias (28). The respiratory tract, the site of more viral infections than any other organ system, merits a vigorous search for viral participation in oncogenesis. Indeed, viral carcinogenesis has been postulated for many human tumors, but earlier techniques yielded inconclusive evidence. Viral human oncogenesis might now again be investigated by a formal search for unique sequences similar to those of counterpart oncogenic viruses of animals.

A recent paper (29) reported that grade 1 infiltrating ductal cancers had 23% positivity, Grade 2 34% and Grade 3 38%. Other types of non-cancerous breast pathology, a history of which is considered a risk factor for subsequent breast cancer, had 24% positivity. Furthermore, male gynecomastia specimens were 19% positive whereas male breast cancers were 62% positive. These graded progressions were considered significant in supporting the possibility of causation.

Fig. 1 Fluorescent in situ hybridization of a 2.7-kb env-ltr probe in env-positive human breast cancer cells cultured from a pleural effusion.
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