Viruses and human breast cancer

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There are well-established risk factors for breast cancer, most of which relate to estrogens and growth hormones in females. These include early-age menarche, late-age menopause, postmenopausal obesity and use of hormone therapy. However, these factors do not account for the sixfold difference in breast cancer incidence and mortality between countries and the fact that these differences dramatically lessen after migration; nor do they account for male breast cancer. Accordingly, hormone-responsive viruses have become major suspects as etiological agents for human breast cancer. Human papillomaviruses, mouse mammary tumor virus and Epstein–Barr virus are the prime candidate viruses as causes of human breast cancer. Human papillomaviruses and the mouse mammary tumor virus have hormone responsive elements that appear to be associated with enhanced replication of these viruses in the presence of corticosteroid and other hormones. This biological phenomenon is particularly relevant because of the hormone dependence of breast cancer. Viral genetic material for each of these candidate viruses has been identified by polymerase chain reaction in breast tumors but rarely in normal breast tissue controls. Pooled data from controlled studies show substantial odds ratios for the presence of viral genetic material in breast tumors compared with normal controls. These and additional data provide substantial, but not conclusive, evidence that human papillomavirus, the mouse mammary tumor virus and Epstein–Barr virus may have a role in the etiology of human breast cancer. If conclusive evidence for a role of these viruses in breast carcinogenesis can be developed, there is a practical possibility of primary prevention.

John Bittner and others at the Jackson Laboratories in Maine, ME, USA, discovered a factor transmitted by mothers’ milk that caused mammary tumors in both field and experimental mice 70 years ago [1]. This was later shown to be the mouse mammary tumor virus (MMTV). This discovery raised the obvious question: could similar viruses cause human breast cancer? An intense period of research followed in the post World War II decades, and a number of inconclusive observations were made. These included the identification of electron microscopic images of viruses in human mother’s milk that appeared to have similar characteristics to MMTV [2] and the detection of MMTV glycoprotein-52 cross-reactive proteins in tumor tissue of women with breast cancer [3]. Unfortunately, these and other studies [4–6] proved highly controversial – the electron microscopic images being likened to milk aggregates and the cross-reactive proteins being found both in healthy and in breast tumor-bearing women, albeit with different frequencies, suggesting that the antibodies used might cross-react with cellular proteins [7].

Further studies in the 1980s investigated the presence of antibodies cross-reactive to MMTV antigens in patient’s sera. Again, although some studies reported a positive correlation [8,9], these studies were highly controversial [10,11] since the correlations shown were far from absolute. Antigen- and antibody-based studies were gradually replaced by molecular biological investigations during the 1980s. Initially it seemed easy to demonstrate DNA sequences cross-hybridizing with MMTV sequences in human breast tumors [12], but it was soon realized that the normal human genome also contained similar sequences [13,14]. These sequences were later designated as human endogenous retroviruses (HERVs) and shown to make up more than 1% of the human genome [15,16]. Prior to nucleic acid sequencing, it was difficult to distinguish between MMTV and HERV sequences and many scientists left the field to pursue other interests.

A quiet and largely unrecognized change to this climate of disinterest began in the early 1990s. Human papilloma virus (HPV) sequences were identified in human breast tumors and it was shown that some of these women with HPV-associated breast cancer also had the same high-risk HPV type associated cervical cancer [17,18]. Epstein–Barr viral (EBV) sequences were identified in human breast tumors but rarely in normal
breast tissue controls [19]. In 1995, a research group led by Beatriz Pogo at the Mount Sinai Hospital, NY, USA, identified sequences from the MMTV envelope (env) region in human breast tumors that had low homology to human HERV env gene sequences [20]. This allowed the identification of an MMTV-like virus in humans that could be distinguished from the thousands of HERV sequences in the normal human genome. The term MMTV-like virus will be used in this article, rather than terms used by others, such as human mammary tumor virus or human homolog of the mouse mammary tumor virus. This is because the MMTV-like virus genetic material that has been identified in human breast cancers and other tissues may well be the same well-known MMTV but with minor variations in its genetic sequences. In addition, the term MMTV is so well established, that readers will immediately understand the topic under discussion.

Additional viruses have recently been added to the seemingly endless list of viral suspects for human breast cancer. These include bovine leukemia virus (BLV) and human herpes virus-8 (HHV-8; Kaposi’s sarcoma virus). Gertrude Buerhing of the University of California at Davis, CA, USA, has identified both antibodies to BLV in sera of women with breast cancer plus BLV gene sequences in human breast tumors [21,22]. She postulates that BLV could account for some rare human breast cancers. HHV-8 was also among a group of viruses recently identified in breast cancer in Taiwanese women [23].

Making sense of seemingly incoherent causal evidence

There are three striking features about breast cancer [24]. These are:

- Breast cancer is over 100-times more common in women than men;
- There are over sixfold differences in incidence and mortality between high- and low-risk populations (e.g., the USA compared with Japan and South Korea);
- These differences rapidly lessen to equalize within two generations of migration from low- to high-risk countries.

Other features of breast cancer include [24]:

- High birth weight increases risk (× 1.5 for highest quartile of birth weights);
- Adult height increases risk (× 1.5 for highest quartile of heights);
- Obesity reduces risk in premenopausal women but increases risk in postmenopausal women;
- High gland mass as distinct from breast size, which can be shown by high-density mammograms, increases risk fourfold;
- Early-age menarche and late-age menopause increase risk (× 2 at extremes);
- Late-age first pregnancy increases risk (× 3 after the age of 40 years);
- Family history increases risk (× 2 for first-degree relative);
- Alcohol consumption slightly increases risk;
- Current use of oral hormone contraceptives increases risk (× 1.2) and current hormone replacement therapy increases risk (× 1.7);
- Mutations in BRCA1 and BRCA2 genes increase risk in families but account for less than 5% of all breast cancers;
- Cancer in one breast increases risk of cancer in the other breast fourfold;
- Full-term pregnancy reduces risk (× 2) compared with nulliparous.

There are plausible explanations for much of this evidence. There is a critical role for estrogens and other hormones in the etiology of breast cancer [25]. Basically, no estrogens, no breast cancer, as shown by the low incidence of breast cancer among women who have been surgically castrated. This role for estrogens and growth hormones is the likely explanation for the risk associated with high birth weight, height and obesity in postmenopausal women (adipose-sourced estrogens suppresses ovarian estrogen in premenopausal women, but increases circulating estrogens in postmenopausal women), early-age menarche and late-age menopause, use of hormone contraceptives and hormone replacement therapy. Alcohol increases circulating estrogens. However, while it is possible that high concentrations of estrogens are carcinogenic, the role of estrogens as carcinogenic cofactors has greater biological plausibility.

The increased risk of late-age pregnancy has been attributed to the protection offered by specialization of breast milk cells associated with early-age pregnancy, making them less likely to begin to proliferate uncontrollably. The reduction of risk associated with full-term pregnancy compared with nulliparous women has also been attributed to specialization of breast milk cells. In mice, exactly the opposite is true: pregnancy increases risk of mammary tumors since expression of MMTV is stimulated by pregnancy hormones and
increased amounts of MMTV increase the chance of infection and integration of MMTV in the vicinity of a cellular proto-oncogene leading to its activation. The reason for this dichotomy may be that in the inbred mouse models studied to date, MMTV is the primary cause of breast cancer and is produced at high levels in infected animals. In contrast, if MMTV plays a role in the etiology of human breast cancer it may not be the predominant cause. Furthermore, the amount of MMTV-like virus produced in humans is low compared with that detectable in mice. Thus, it is unlikely that the presence or absence of hormone will greatly affect the reinfection rate.

There has been an immense research effort devoted to a possible role for diet, particularly fats, in breast cancer. This effort was based on sound experimental evidence that showed that increased fats are associated with increased risk of mammary cancer in laboratory rodents [26], in combination with observations on dietary changes in migrants from countries with a low breast cancer risk to those with a high risk. At first glance these studies suggest that diet has only a minimal effect on breast cancer risk [27]. However, the lack of correlation between fat consumption and breast cancer risk may be misleading because most studies have involved Western women. Even Western women with the lowest food consumption consume higher levels of energy and fats than most women from Asian populations; for instance, Indonesian energy and fat consumption levels are 50 and 25%, respectively, of levels in the USA. In case–control studies of food consumption and breast cancer incidence in Asian countries such as Indonesia, there is a clear association between food consumption patterns and breast cancer risk [28].

Diet probably exerts an influence on breast cancer risk through its influence on hormone metabolism. There is consistent epidemiological evidence that patterns of estrogen metabolism and serum estrogen levels are dependent on diet and that these are associated with breast cancer risk [29–31]. Urinary and serum estrogen and testosterone levels are over 50% higher in Western compared with Asian women. Serum sex hormone levels have been shown to be directly correlated with breast cancer risk in postmenopausal women with a sixfold relative risk between highest and lowest hormone level quartiles of the study population [32].

Changes in diet and increased circulating hormone levels in migrants from countries with a low breast cancer risk to those with a high risk and subsequent increased incidence of breast cancer may be an epigenetic phenomenon. Epigenetics refers to heritable changes in gene expression that occur without a change in DNA sequences [33]. Epigenetic abnormalities can be causal of cancers and some reports have linked a common polymorphism in the methylenetetrahydrofolate reductase gene to altered DNA methylation patterns in response to diet, alcohol consumption and hormone replacement therapy, with an apparent increase in the incidence of breast cancer in some populations [34–36].

There have been many reviews of the epidemiology and risk factors associated with breast cancer [37]. While the above risk factors almost certainly account for some of the increased risk for breast cancer among migrants from low- to high-risk countries, they do not account for the majority of the increased risk [38]. Nor, as stated above, do these risk factors account for breast cancer in males. Hence, the renewed interest in hormone-regulated viruses as causal agents.

A diet, hormone-related, viral etiology of breast cancer hypothesis offers a possible explanation for the heterogenous histological characteristics of human breast cancer, which is in contrast to the homogenous histological characteristics of MMTV-associated mouse mammary tumors. However, a subset of human breast cancers may have similar histology to mouse mammary tumors. It may be that three or more viruses, each known to have carcinogenic properties in either animals or humans, could be associated with human breast cancer. Such a possibility is made more plausible because the replication of two of these viruses, MMTV and HPV, are known to be enhanced by sex and growth hormones. These hormones are obligatory for breast carcinogenesis. However, it is only in very recent times that limited evidence has emerged in support of this histopathological speculation [39,40].

**HPVs & breast cancer**

**HPV viral genetic material is present in breast tumor tissues & cells but rarely in normal breast tissues**

The recent identification of HPVs by de Villiers and colleagues, Damia and colleagues and Kan and colleagues in breast tumors [39,41,42], has established HPVs as strong candidate oncoviruses for breast cancer. de Villiers and colleagues have shown that breast nipple tumors in which HPVs have...
been identified have histological characteristics typical of HPV-associated human cancers (such as the cervix) [39]. de Villiers and colleagues identified a range of HPV types in 25 out of 29 samples of breast carcinoma [39]. Damin and colleagues identified HPV types 16 and 18 in 24.75% of breast tumors (n = 105) but not in normal controls [41]. Kan and colleagues identified HPV-18 in 48% of DNA extracted from breast tumors [42].

The presence of HPV DNA genes in breast tumors has been identified in ten out of 12 studies, as shown in Table 1, including every study conducted since 1999. Normal breast tissue controls were available for four of these studies. In these four studies, there were 215 cases, and HPV gene sequences were identified in 51 (23.7%). There were 89 controls, and HPV sequences were identified in one (1.1%). This is an adjusted (for sample size) odds ratio (OR) of 7.18 (1.81–28.47).

In contrast to cervical cancer, HPVs are difficult to detect in breast cancer specimens. For example, in our own study, HPVs could not be detected in fresh frozen specimens using standard PCR, but could be detected with added amplification of DNA [42]. It seems likely that these difficulties may be due to low HPV DNA copy numbers in breast cancer.

Table 1. Results of studies into the presence of HPV genetic material in human breast cancer.

<table>
<thead>
<tr>
<th>First author (year)</th>
<th>Region</th>
<th>Specimen</th>
<th>Assay</th>
<th>Cases/HPV+ (%)</th>
<th>Controls/HPV+ (%)</th>
<th>OR (CI)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controlled studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yu (1999)</td>
<td>China</td>
<td>Fixed</td>
<td>PCR</td>
<td>34/15 (44.1%; HPV-33)</td>
<td>2/0 (0.0%)</td>
<td>3.97 (0.18–89.00)</td>
<td>[117]</td>
</tr>
<tr>
<td>Yu (1999)</td>
<td>Japan</td>
<td>Fixed</td>
<td>PCR</td>
<td>18/3 (16.6%; HPV-16, -33)</td>
<td>18/1 (5.6%)</td>
<td>3.40 (0.32–36.27)</td>
<td>[117]</td>
</tr>
<tr>
<td>Damin (2004)</td>
<td>Brazil</td>
<td>Fixed</td>
<td>PCR</td>
<td>101/25 (25%; HPV-16, -18)</td>
<td>41/0 (0.0%)</td>
<td>27.67 (1.64–466.13)</td>
<td>[41]</td>
</tr>
<tr>
<td>Tsai (2005)</td>
<td>Taiwan</td>
<td>Frozen</td>
<td>PCR</td>
<td>62/8 (12.9%)</td>
<td>28/0 (0.0%)</td>
<td>8.89 (0.50–159.63)</td>
<td>[23]</td>
</tr>
<tr>
<td><strong>Noncontrolled studies</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Lonardo (1992)</td>
<td>Italy</td>
<td>Fixed</td>
<td>PCR</td>
<td>17/3 (19.4%; HPV-16)</td>
<td>–</td>
<td>–</td>
<td>[17]</td>
</tr>
<tr>
<td>Bratthauer (1992)</td>
<td>USA</td>
<td>–</td>
<td>PCR</td>
<td>28/0 (0.0%; HPV-6, -11, -16, -18)</td>
<td>–</td>
<td>–</td>
<td>[118]</td>
</tr>
<tr>
<td>Wrede (1992)</td>
<td>England</td>
<td>–</td>
<td>PCR</td>
<td>80/0 (0.0%)</td>
<td>–</td>
<td>–</td>
<td>[119]</td>
</tr>
<tr>
<td>Gulpalkrishna (1996)</td>
<td>India</td>
<td>Frozen</td>
<td>PCR</td>
<td>30/0 (0.0%; HPV-16, -18)</td>
<td>–</td>
<td>–</td>
<td>[120]</td>
</tr>
<tr>
<td>de Villiers (2005)</td>
<td>USA</td>
<td>Fixed</td>
<td>PCR</td>
<td>29/25 (86%; HPV-6, -11 most prevalent)</td>
<td>–</td>
<td>–</td>
<td>[39]</td>
</tr>
<tr>
<td>Kan 2005</td>
<td>Australia</td>
<td>Frozen</td>
<td>PCR</td>
<td>24/50 (48%; HPV-18)</td>
<td>–</td>
<td>–</td>
<td>[42]</td>
</tr>
</tbody>
</table>

CI: Confidence interval; HPV: Human papillomavirus; OR: Odds ratio; PCR: Polymerase chain reaction.
Malignant transformation of normal breast epithelial cells by HPVs

Estrogens and other hormones activate the HPV promoter and facilitate the immortalization of HPV-infected normal human breast epithelial cells [45]. For full malignant transformation of immortalized cells, accumulation of cellular changes by long-term passaging is necessary [45].

Presence of HPV sequences in breast cancers has a typical malignant phenotype/morphology

As outlined above, de Villiers and colleagues have observed that HPVs are present in cancers occurring in human nipple milk ducts and that these cancers have the typical histological features of HPV-induced human cancers (cervical cancer) [39]. The features identified include epithelial hyperplasia and the suggestive appearance of koilocytosis. In addition, squamous metaplasia of lactiferous ducts and shedding of metaplastic elements into lactiferous ducts and sinuses were noted.

Methods of transmission of HPV

The global epidemiology of HPV-associated cervical cancer is now well known, but there are no epidemiological studies of possible HPV-related breast cancer.

The means by which HPVs are transmitted to the breast are not known. HPVs are known to be released when the cornified envelope of cells desquamate and accordingly, HPVs can be transmitted by skin-to-skin contact, as well as by sexual activity [46]. In a recent Finnish study, a range of HPVs that are high risk for breast cancer were identified in all family members, including infants [47]. Accordingly, transmission of HPVs is not confined to sexual activity.

In addition, HPV sequences of the same type (HPV-16), have been identified in breast tumors that occur in women with HPV-associated cervical cancer [18,44]. Therefore, it is possible that HPVs may be transmitted by hand from the female perineum to the breast, which could occur during sexual activities or even showering or bathing.

Hormones & HPVs

Breast cancer is estrogen dependent [25]. Accordingly, the presence of a virus in breast cancers that is also influenced by hormones is of special interest. Almost all of the investigations related to hormones and HPVs have been concerned with cervical cancer. While the precise mechanisms of HPV-related cervical oncogenesis are not known, the transforming genes (E6 and E7) play a critical role and it is clear that estrogens synergize with high-risk HPV oncogenes to cause human cervical cancer [48]. In addition, the regulatory region of HPV-16 genomic DNA contains sequences that are responsive to glucocorticoid hormones [49]. Finally, high estrogen affects breast epithelial cell proliferation, thereby playing an added role in breast cancer.

Interpretation of the HPV- & breast cancer-related data

It is biologically plausible to consider any virus that is a proven carcinogen in a specific human cancer to be a prime suspect when this virus is identified in other types of cancer. HPVs are such viruses.

In addition, each of the conventional causal criteria have almost been met [50], namely: HPV genetic material has been consistently identified in breast tumors but rarely in normal controls; HPV immortalizes normal human breast epithelial cells in vitro; a possible means of transmission has been identified; the same HPV type occurs in breast and cervical cancer in the same patients; and the mechanisms of HPV as an oncovirus are broadly understood.

MMTV & breast cancer

MMTV is the etiologic agent of mammary tumors in field and experimental mice [51,52]. MMTV is transmitted both through mouse mother's milk (exogenous transmission) and through the mouse germline (endogenous transmission).

MMTV expression is hormone responsive, resulting in increased amounts of exogenous MMTV being produced during pregnancy and lactation [51,52].

Hormone stimulation of MMTV occurs via a hormone response element (HRE), which is a short base pair sequence to which the hormone receptor binds and enhances transcription [53,54]. Glucocorticoid, estrogen, progesterone and prolactin HREs are present in MMTV-like human viral long terminal repeats. There are complex interactions between these hormones and MMTV. For example, estrogen enhances the responsiveness of MMTV to glucocorticoids.

Studies on human gestational breast cancer that arises during or immediately after pregnancy and is associated with a poorer prognosis than other types of breast cancer, have shown that MMTV-like env gene sequences can be detected in 62% of the studied samples compared with 30–38% of randomly selected breast.
One possible interpretation of these data would be that expression of MMTV-like sequences present in the human genome is stimulated by pregnancy hormones via the HRE element, leading to increased viral load and reinfection. Indeed, the same authors have also reported MMTV-specific RNA expression in human mammary tumors carrying such MMTV-like sequences [56].

**MMTV-like viral genetic material is present in breast tumor tissues & cells but rarely in normal breast tissues**

MMTV-like envelope gene sequences have been identified in ten new studies, conducted in four independent laboratories (Table 2); MMTV-like sequences in breast tumors were not detected in three published studies (Table 2). All of these studies used primers identified by the Beatriz Pogo group as having low homology to human endogenous retroviral sequences [20]. Normal breast tissue controls (specimens from cosmetic surgery and benign nonproliferative breast conditions) were available for five of these studies (Table 2). In these five studies, there were 612 breast cancer cases; MMTV-like gene sequences were identified in 222 (36.3%). There were also 369 controls, and MMTV-like sequences were identified in six (1.7%). This is an adjusted (for size of study) OR of 27.55 (confidence interval: 12.26–61.91).

Wang and colleagues identified MMTV mRNA in breast cancer specimens but not in normal breast tissues [56]. Ford and colleagues have confirmed these observations using reverse transcriptase in situ PCR to detect MMTV env gene RNA transcripts within cancerous breast epithelial cells in 78% of MMTV DNA positive tumors (n = 33) [57]. MMTV-like RNA has never been detected in normal breast epithelial tissues.

<table>
<thead>
<tr>
<th>First author (year)</th>
<th>Region</th>
<th>Specimen</th>
<th>Assay</th>
<th>Cases/ MMTV+ (%)</th>
<th>Controls/ MMTV+ (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang (1995)</td>
<td>USA</td>
<td>Fixed/frozen</td>
<td>PCR</td>
<td>314/121 (38.5%)</td>
<td>107/2 (1.8%)</td>
<td>[20]</td>
</tr>
<tr>
<td>Etkind (2000)</td>
<td>USA</td>
<td>Frozen</td>
<td>PCR</td>
<td>73/27 (37.0%)</td>
<td>35/0 (0.0%)</td>
<td>[121]</td>
</tr>
<tr>
<td>Melana (2001)</td>
<td>Italy</td>
<td>Fixed</td>
<td>PCR</td>
<td>106/32 (30.1%)</td>
<td>106/1 (0.9%)</td>
<td>[122]</td>
</tr>
<tr>
<td>Melana (2002)</td>
<td>Argentina</td>
<td>Fixed</td>
<td>PCR</td>
<td>74/23 (31%)</td>
<td>10/1 (10%)</td>
<td>[123]</td>
</tr>
<tr>
<td>Ford (2003)</td>
<td>Australia</td>
<td>Fixed</td>
<td>PCR</td>
<td>45/19 (42.2%)</td>
<td>111/2 (1.8%)</td>
<td>[124]</td>
</tr>
<tr>
<td>Ford (2004)</td>
<td>Australia</td>
<td>Fixed</td>
<td>In situ PCR</td>
<td>33/26 (78.8%)</td>
<td>20/0 (0.0%)</td>
<td>[57]</td>
</tr>
<tr>
<td>Zangen (2002)</td>
<td>USA</td>
<td>Fixed tumor</td>
<td>PCR</td>
<td>18/0 (0.0%)</td>
<td>–</td>
<td>[58]</td>
</tr>
<tr>
<td>Wang (2003)</td>
<td>USA</td>
<td>Fresh frozen/fixed tumor</td>
<td>PCR</td>
<td>29/18 (62%)</td>
<td>–</td>
<td>[55]</td>
</tr>
<tr>
<td>Witt (2003)</td>
<td>Austria</td>
<td>Fresh frozen tumor</td>
<td>PCR</td>
<td>50/0 (0%)</td>
<td>–</td>
<td>[59]</td>
</tr>
<tr>
<td>Mant (2004)</td>
<td>England</td>
<td>Fresh frozen tumor</td>
<td>PCR</td>
<td>44/0 (0%)</td>
<td>–</td>
<td>[60]</td>
</tr>
<tr>
<td>Levine (2004)</td>
<td>Tunisia</td>
<td>Fixed tumor</td>
<td>PCR</td>
<td>38/28 (73.7%)</td>
<td>–</td>
<td>[125]</td>
</tr>
<tr>
<td>Etkind (2004)</td>
<td>USA</td>
<td>Fixed tumor</td>
<td>PCR</td>
<td>12/6 (50%)</td>
<td>–</td>
<td>[126]</td>
</tr>
</tbody>
</table>

**Table 2. Results of studies into the possible role of MMTV-like viruses in human breast cancer.**

MMTV: Mouse mammary tumor virus; PCR: Polymerase chain reaction.
cells sourced from cosmetic surgery. This in situ PCR-based study demonstrates the location of the MMTV-like env transcripts in breast cancer cells and not in surrounding lymphocytes or normal breast epithelial cells [57].

The identification of MMTV-like sequences using PCR techniques is difficult and some workers have been unable to identify any MMTV sequences in breast tumors [58–60]. While the reasons are not clear, it is possible that differences in fresh and fixed specimens plus variations in techniques influence the outcome of different studies.

Liu and colleagues have amplified the whole proviral structure of MMTV-like virus, including the env LTR, LTR gag, gag-pol and pol-env, sourced from each of two human breast carcinomas that were MMTV env-positive [61]. This 9.9 kb provirus was 95% homologous to MMTV. It displayed typical features of a replication competent virus, plus the open reading frame for the superantigen and the glucocorticoid responsive element. Liu and colleagues argued that because these MMTV-like sequences are virtually undetectable in normal tissues and because they maintain open reading frames, they are most probably of exogenous origin [61]. However, recent work by Szabo and colleagues, who were able to detect MMTV-like sequences in a wide variety of normal human tissues, has challenged this view, and suggests that endogenous MMTV-like sequences may indeed exist [62].

**Infectivity of MMTV in humans**

After MMTV is ingested by newborn mice in mother's milk, it first infects B lymphocytes in intestinal Peyer's patches. Infection of B cells by MMTV results in the expression of an MMTV-encoded superantigen, which stimulates a large proportion of the T-cell repertoire of the mouse, in turn stimulating the expansion of B cells. It is thought that the virus is then maintained in a long-lived cell of lymphocytic lineage before circulating and infecting mammary epithelial cells at a later time point [63].

Over 20 years ago, Hilkens and coworkers showed, using genetic methods, that the MMTV receptor is located on mouse chromosome 16 [64]. Almost 10 years later, Bolander and Blackstone, using biochemical methods, demonstrated that MMTV binding activity could be found in virtually every tissue, but was upregulated in the mammary gland [65]. More recently, the laboratory of Susan Ross reported the cloning of two genes encoding receptors for MMTV; MTVR, which is located on chromosome 19 [66], and the murine transferrin receptor located on chromosome 16 [67]; however, only the latter gene is able to confer susceptibility to infection by MMTV upon cells that are normally refractive to infection. It is possible that the murine transferrin receptor is one of a number of molecules that could function as a receptor, or that it represents a co-receptor for MMTV. Such an explanation would be supported by the apparently contradictory findings that the human transferrin receptor (TfR1) cannot function as a receptor [67], but that MMTV can efficiently infect human breast epithelial cells [68].

Although no evidence has yet been presented for experimental infection of human lymphocytes with MMTV, the presence of MMTV-like sequences in circulating lymphocytes in patients with breast cancer, as well as in intestine lymphoid tissue, has been reported [69,70]. Recent studies by the Garry group in Louisiana, USA, have confirmed that MMTV sequences are present in human lymphocytes and breast tissues, as well as a range of other species (including monkeys and cats) in addition to mice and other rodents [62].

MMTV is a cell-associated virus in mice. This could also account for its B-type morphology with preformed cores or capsids – allowing it to potentially spread from cell to cell. Even in inbred mouse models it is difficult to find large amounts of MMTV in tissues other than the mammary gland and spleen. This may account for the difficulties in detecting large amounts of virus in strictly outbred human populations, and would argue for the involvement of MMTV in only a subset of mammary tumors. However, the ability to infect human cells is a prerequisite for such a role, and although much indirect evidence has accumulated over the years, the recent direct demonstration of experimental infection of human cells by MMTV, together with the identification of MMTV–human genome junctions in two independently infected human cell lines of independent origin [68], provide the necessary framework in which to pursue this hypothesis further. The next step is to seek to identify an MMTV-like integration site in human breast cancer DNA.

**Endogenous human retroviruses are present but distinct from MMTV**

Approximately 30–50 copies of the HERV-K are present in the human genome. HERV-K is approximately 57% homologous to MMTV...
over the full viral sequence [61], with a typical retroviral genomic organization including gag, pol and env genes. Work carried out in the 1990s by the Lowers’ group demonstrated expression of HERV-K in a number of human tumors [71–73], and based upon this, Wang-Johanning and colleagues have surveyed HERV-K env expression in a panel of 55 breast carcinomas and 75 normal breast tissues both by RT-PCR as well as by in situ hybridization [74]. HERV-K expression was detectable in 45% of breast carcinoma samples, in 18% of samples of apparently normal breast tissue taken from patients with breast cancer, but never in normal breast tissue from healthy patients. The results of the in situ analysis suggested that the positive signals in apparently normal tissue from breast cancer patients might be the result of previously undetected transformed cells. Sequencing of selected HERV-K clones revealed not only a greater than 97% sequence homology to HERV-K102 env but additionally, that there were no stop codons in the entire env reading frame, suggesting that these proteins could be biologically active.

However, it is not clear whether the expression of HERV-K and other HERV family members is a result of activation during the tumorigenic process or a cause of the tumorigenic process. The lack of an exogenous, transmissible HERV-K argues for a similar role for this family of provirus to the majority of endogenous MMTV sequences in mice. These represent ancestral infection events and, with only a few exceptions, cannot give rise to infective virus [51]. However, such endogenous sequences may represent a reservoir for recombination and/or complementation with other defective viral copies, for example by contributing to the formation of pseudotypes.

The recent identification of sequences highly similar to MMTV in normal human lymphocytes, breast cancer and normal breast tissues by Szabo and colleagues [62] suggest that these may be endogenous MMTV-like sequences, as is seen in the mouse model.

Detection of antibodies to MMTV-like viruses & MMTV antigens in tumors from breast cancer patients & controls

Following development of molecular techniques for the identification of MMTV and other viruses, interest in studies on MMTV-like antigens and the detection of MMTV-reactive antibodies lapsed, and many of the early studies are regarded as unsubstantiated by some observers [75]. However, interest in the detection of MMTV antigens as a marker in human cancer was rekindled by the observation that MMTV p14 is translocated into the nucleoli in some human breast cancer specimens but not in normal breast tissue controls [75].

In a recent investigation, no MMTV-specific antibodies were identified in women with breast cancer [76]. This observation challenges the concept of MMTV as a possible cause of human breast cancer. However, there is a possible explanation: if most newborn human infants are exposed to MMTV via colostrum or breast milk, there may be a substantially reduced immune response, as appears to be the case with newborn mice [77]. It appears that virtually all newborn human infants are exposed to MMTV via colostrum or milk soon after birth [78], despite the apparent evidence to the contrary [79]. Thus, it may be that human newborn infants also have a reduced immune response to MMTV.

An MMTV-like superantigen has also been identified in human breast cancer [80]. This has certain parallels with the mouse model. As mentioned previously in the mouse, expression of this superantigen is required for the stimulation of T lymphocytes, allowing the amplification of virus-infected cells and ultimately pathogenesis [81,82].

Transformation of normal breast epithelial cells by MMTV

MMTV, like all retroviruses, integrates fairly randomly into the genome of infected cells. Integration may be preferentially found in open chromatin, and some evidence has been presented for a certain preference for the promoter region of genes for murine leukemia viruses or the body of genes for HIV [83,84]. If MMTV integration takes place in the vicinity of a cellular proto-oncogene, the expression of the proto-oncogene can be upregulated. Indeed, analysis of a large number of murine mammary tumors has revealed that in almost all cases, MMTV integration can be found either up or downstream of cellular proto-oncogenes of the int family. The expression of the cellular proto-oncogene is then upregulated as a result of the influence of the strong MMTV transcriptional enhancer.

To date, it has not been possible to experimentally reproduce this transformation process in cell culture due to the very large number of integration events required before, by chance, the expression of a cellular proto-oncogene will become deregulated. Furthermore, the overexpression of just one
Viruses and human breast cancer – PERSPECTIVE

gene is not sufficient to lead to the fully transformed phenotype; rather, a number of genes need to be deregulated. However, for many years it has been suggested that MMTV itself may encode a protein that can also play a role in part of this transformation procedure, and recent evidence from the laboratory of Susan Ross supports this notion. This group was able to demonstrate that the envelope protein of MMTV contains an immunoreceptor tyrosine-based activation motif that, when expressed in normal murine mammary cells growing in three-dimensional cultures, results in many changes associated with transformation [85].

However, while these results have been achieved with MMTV, they have not yet been demonstrated for human-sourced MMTV-like virus

Breast cancers carrying MMTV-like sequences have a typical malignant phenotype/morphology

It has been shown that 42.4% (n = 66) of human invasive breast tumor specimens have some similar histological characteristics to MMTV-induced mouse mammary tumor specimens [40]. However, there were no correlations between the presence and absence of MMTV sequences in the human breast tumors and similarity to mouse mammary tumors. It is unusual for the whole of the human breast tumor to show a similar histology to the mouse tumor, with similarities being mostly confined to only small areas of the human tumor.

Infectivity of MMTV in humans

Human cells can be infected by MMTV [68], and this is dependent on the presence of a functional, wild-type viral envelope. However, such infection has yet to be achieved with human-sourced MMTV-like virus.

Methods of transmission of MMTV-like virus

In mice, it is known that MMTV is transmitted exogenously through mouse mother's milk and endogenously via the mouse germline. Although similar, there are a number of different strains of exogenous MMTV and early studies demonstrated that their ability to cause mammary cancer in mice varied and also depended on the inbred strain of mouse that was infected with the virus.

MMTV-like particles have been observed in human milk from women with breast cancer and women with a family history of breast cancer [2]. However, these observations have not been confirmed or replicated and are regarded by many as controversial. There is seemingly consistent evidence that breast-fed babies are at no higher risk of developing breast cancer than nonbreast-fed babies and, at least among epidemiologists, there is a consensus that milk-borne viruses are not associated with human breast cancer [79]. Nevertheless, it is well established that the human retroviruses HIV and human T-lymphotropic virus can be transmitted by infected milk [86], and evidence is emerging that the epidemiological evidence referred to above may not, in some respects, be sound [87]. This is because it has been realized that virtually all epidemiological studies into breast feeding have been based on questionnaires, and mothers respond in varying ways depending on various understandings of the meaning of breast feeding. Current data, based on direct observation, suggests that it is possible that 90–100% of newborn babies are ‘exposed’ to colostrum or breast milk, despite failure of some mothers to continue breast feeding [78]. Accordingly, transmission of MMTV by human breast milk to newborn infants is possible.

An intriguing possibility is the spread of MMTV-like virus from human to human by skin surface contact. This speculative hypothesis is based on the knowledge that MMTV virions are stably maintained in sebaceous glands of mice [88].

Epidemiologically it has been suggested that the worldwide distribution of MMTV-like virus gene sequences in breast cancer in human populations parallels the distribution of the MMTV-carrying common house mouse (Mus domesticus) [89]. Continuous zoonotic (animal to human) transmissions of MMTV are a possibility. Airborne particles of mouse materials are common household allergens and therefore are theoretical sources of transmission of MMTV from mice to humans [90]. Transmission of MMTV by human ingestion of cereal and other food contaminated by mouse fecal material is also a speculative possibility. These possibilities are enhanced by the knowledge that adult laboratory personnel exposed to MMTV as part of their work appeared to experience MMTV-specific serological responses compared with control groups [91]. Anecdotally, a female laboratory worker exposed to mouse mammary tumors gave four negative antibody responses to MMTV. She then developed positive MMTV serum antibodies followed by breast cancer [92]. However, given the controversy with respect to analyses of MMTV antibodies in humans, these observations may not be reliable.
Interpretation of MMTV-related data
The repeated identification of MMTV-related genetic material in human breast tumors, in contrast to the rare identification in normal breast tissue controls, is supportive of a role for MMTV-like viruses in human breast cancer.

Similarly, the consistent identification of MMTV-related antibodies in sera and breast cyst fluids from patients with breast cancer compared with normal controls, plus the identification of MMTV-related antigens in breast cancers compared with controls is also supportive of a role for MMTV-like viruses in breast cancer. However, the possibility that some of this data may be misleading due to cross reactions cannot be excluded.

Additional supportive evidence is the identification of an MMTV-related superantigen in human breast cancers; the observation that the histological characteristics of some human breast cancers may be similar to MMTV-related mouse mammary tumors; that expression of MMTV in normal human breast epithelial cells results in phenotypic changes associated with transformation; that human cells can be infected by MMTV; and that MMTV can integrate into the human genome. Integration of MMTV into the genome of mice, and subsequent activation of proto-oncogenes in the vicinity is an early step in mammary carcinogenesis in rodents.

However, others have strongly argued that an MMTV-like agent or virus is an unconvincing etiological agent for human breast cancer [60]. Mant and Cason challenge the proponents of MMTV-related human breast carcinogenesis to explain [60]:

- How MMTV can infect *Homo sapiens* given that human cells lack the necessary transferring receptor for MMTV and that MMTV is not an endogenous human retrovirus;
- Why immunosuppression does not increase the risk of breast cancer, as is the case with other human oncoviruses;
- Why human breast feeding does not predispose daughters to breast cancer, as is the case with mice;
- Why pregnancy is protective against the risk of human breast cancer whereas the opposite is true for MMTV-caused mouse mammary hyperplasia.

These seemingly valid points have been vigorously answered by Pogo and Holland, the originators of the recent interest in MMTV-like viruses and human breast cancer [93]. They and others argue:

- That MMTV has been shown to be able to infect human cells [68];
- Four independent laboratories have identified MMTV-like *env* sequences in human breast tumors but rarely in normal control tissues, and that negative findings by others may be due to the use of different techniques;
- That the MMTV-like provirus identified in human breast tumors is likely to be exogenous and not endogenous, primarily because the sequences have been found in breast tumors and rarely in normal breast tissues;
- In humans, early pregnancy is a protective factor against breast cancer, but gestational cancer (breast cancer during pregnancy and for 12 months post partum) is extremely virulent, suggesting that high levels of hormones may be involved;
- That involvement of the immune system in MMTV infections is unique and differentiates this virus from other tumor viruses.

For this review, we suggest an additional speculative theory for the apparent lack of breast cancer in immunosuppressed patients than in the general population [94] – if MMTV infection in humans follows a similar route as in mice and involves an amplification of initially infected antigen presenting cells via expression of a superantigen, then it would make sense that immunosuppression would have a major negative impact on virus amplification and thus on subsequent infection and transformation.

**EBV & breast cancer**
A detailed review of EBV in relation to breast cancer has just been published [95]. EBV may not be directly carcinogenic for breast cancer. Instead, EBV may alter the behavior of already transformed cells so that they acquire a more aggressive phenotype. This hypothesis is supported by the observation that EBV-associated breast cancers are more commonly estrogen receptor-negative and aggressive than other breast cancers [96,97].

EBV genetic material in human breast tumors was first identified by Horiuchi and colleagues in Japanese women [98]. Over 20 studies of EBV and breast cancer have followed. These are listed in Table 3. The results of these studies have been inconsistent. For this reason, we have not summarized the data. This inconsistency may be due to the use of different techniques and the use of formalin-fixed compared with fresh tissues. EBV has been identified most consistently using PCR.
Table 3. Results of studies into the presence of EBV genetic material in human breast cancer.

<table>
<thead>
<tr>
<th>First author (year)</th>
<th>Region</th>
<th>Specimen</th>
<th>Assay</th>
<th>Cases/EBV+ (%)</th>
<th>Controls/MMTV+ (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labreque (1995)</td>
<td>England</td>
<td>Frozen/fixed</td>
<td>PCR/ISH</td>
<td>91/19 (21%)</td>
<td>21/0 (0%)</td>
<td>[19]</td>
</tr>
<tr>
<td>Bonnet (1999)</td>
<td>France</td>
<td>Frozen/fixed</td>
<td>PCR</td>
<td>100/51 (51%)</td>
<td>30/3 (10%)</td>
<td>[96]</td>
</tr>
<tr>
<td>Grinstein (2002)</td>
<td>USA</td>
<td>Fixed</td>
<td>PCR</td>
<td>33/14 (42%)</td>
<td>21/0 (0%)</td>
<td>[127]</td>
</tr>
<tr>
<td>Tsai (2005)</td>
<td>Taiwan</td>
<td>Frozen</td>
<td>PCR</td>
<td>69/28 (45.2%)</td>
<td>6/0 (0%)</td>
<td>[23]</td>
</tr>
<tr>
<td>Perrigoue (2005)</td>
<td>USA</td>
<td>Fixed</td>
<td>PCR</td>
<td>45/0 (0%)</td>
<td>45/0 (0%)</td>
<td>[107]</td>
</tr>
<tr>
<td>Noncontrolled studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gaffey (1993)</td>
<td>USA</td>
<td>Fixed</td>
<td>PCR</td>
<td>35/0 (0%)</td>
<td>–</td>
<td>[128]</td>
</tr>
<tr>
<td>Horiuchi (1994)</td>
<td>Japan</td>
<td>Fixed</td>
<td>PCR</td>
<td>3/2 (66%)</td>
<td>–</td>
<td>[98]</td>
</tr>
<tr>
<td>Lespagnard (1995)</td>
<td>Belgium</td>
<td>Fixed</td>
<td>PCR</td>
<td>10/0 (0%)</td>
<td>–</td>
<td>[130]</td>
</tr>
<tr>
<td>Glaser (1998)</td>
<td>USA</td>
<td>Fixed</td>
<td>ISH</td>
<td>84/0 (0%)</td>
<td>–</td>
<td>[131]</td>
</tr>
<tr>
<td>Chu JS (1998)</td>
<td>Taiwan</td>
<td>Fixed</td>
<td>ISH</td>
<td>60/0 (0%)</td>
<td>–</td>
<td>[132]</td>
</tr>
<tr>
<td>Brink (2000)</td>
<td>Holland</td>
<td>Frozen</td>
<td>PCR</td>
<td>24/5 (21%)</td>
<td>–</td>
<td>[133]</td>
</tr>
<tr>
<td>McCall (2001)</td>
<td>USA</td>
<td>Fixed</td>
<td>PCR</td>
<td>115/2 (2%)</td>
<td>–</td>
<td>[134]</td>
</tr>
<tr>
<td>Finna (2001)</td>
<td>North Africa</td>
<td>Fixed/frozen</td>
<td>PCR</td>
<td>509/162 (32%)</td>
<td>2/0 (0%)</td>
<td>[101]</td>
</tr>
<tr>
<td>Chu PG (2001)</td>
<td>USA     Europe</td>
<td>Fixed/frozen</td>
<td>PCR</td>
<td>48/5 (10%)</td>
<td>–</td>
<td>[104]</td>
</tr>
<tr>
<td>Dadmanesh (2001)</td>
<td>Italy</td>
<td>Fixed</td>
<td>ISH IHC</td>
<td>6/0 (0%)</td>
<td>–</td>
<td>[135]</td>
</tr>
<tr>
<td>Kijima (2001)</td>
<td>Japan</td>
<td>Fixed</td>
<td>ISH</td>
<td>61/0 (0%)</td>
<td>–</td>
<td>[136]</td>
</tr>
<tr>
<td>Deshpande (2002)</td>
<td>USA</td>
<td>Fixed</td>
<td>IHC</td>
<td>43/0 (0%)</td>
<td>–</td>
<td>[137]</td>
</tr>
<tr>
<td>Herrmann (2003)</td>
<td>Germany</td>
<td>Fixed</td>
<td>PCR</td>
<td>59/4 (6.8% positive)</td>
<td>–</td>
<td>[99]</td>
</tr>
<tr>
<td>Xue (2003)</td>
<td>England</td>
<td>Fixed/frozen</td>
<td>PCR</td>
<td>15/6 (40%)</td>
<td>–</td>
<td>[102]</td>
</tr>
<tr>
<td>Murray (2003)</td>
<td>UK</td>
<td>Frozen</td>
<td>PCR</td>
<td>92/19 (21%)</td>
<td>–</td>
<td>[97]</td>
</tr>
<tr>
<td>Thorne (2005)</td>
<td>USA</td>
<td>Fixed</td>
<td>PCR</td>
<td>55/4 (7%)</td>
<td>–</td>
<td>[138]</td>
</tr>
<tr>
<td>Arbach (2006)</td>
<td>France</td>
<td>Frozen</td>
<td>PCR</td>
<td>95/44 (46%)</td>
<td>–</td>
<td>[139]</td>
</tr>
</tbody>
</table>

EBV: Epstein–Barr virus; IHC: Immunohistochemistry; ISH: In situ hybridization; PCR: Polymerase chain reaction.
(in 14 out of 17 studies). This is in contrast to the use of in situ hybridization (ISH) and immunohistochemistry (IHC), most of which have been negative.

It has been strongly argued that infiltrating EBV-infected lymphocytes might explain the presence of EBV in breast tumors [99]. For this reason, strenuous efforts have been made to identify the cellular location of EBV genetic material in breast tumors. Microdissection to isolate cancer cells from lymphocytes and ISH and IHC has been used in combination with PCR to resolve this issue. Labreque and colleagues, Bonnet and colleagues, Arbach, Fina and colleagues, and Xue and colleagues [19,96,100–102] have clearly shown that EBV genetic material is present in many, but not all, cancer cells and to a lesser extent in some infiltrating lymphocytes.

EBV genetic material has been identified in epithelial cells contained in human breast milk in 46% of healthy women [103].

**EBV & clinical characteristics**

Steroid receptor status and grade of breast tumor have been assessed in two studies that identified the presence of EBV sequences in breast tumors [96,97]. In both of these studies there was a significant negative correlation between estrogen receptor expression and the presence of EBV sequences. In addition, in both studies the presence of EBV sequences was significantly associated with high tumor grade. An association between the presence of EBV genetic material and higher breast cancer grade has also been observed by Fina and colleagues [101] and Chu and colleagues [104].

**Serology**

In the only published study of classical serological markers in breast cancer patients there were no differences in seropositivity or EBV immunoglobulin levels between cases and controls [105].

**Immortalization of normal breast epithelial cells by EBV**

Transfection of the p31 subfragment of EBV DNA stimulates the growth of normal human breast epithelial cells [102].

The mechanism for EBV oncogenesis is related to viral proteins, which usurp cellular pathways that promote cell growth and survival while impairing antiviral immune responses, including lymphocytes [106].

**Interpretation of EBV-related data**

The association of EBV with human breast cancer remains controversial despite the well-documented presence of EBV genetic material in up to 50% of breast tumors but rarely in normal breast tissue controls (Table 3). This controversy is due to the failure of some investigators to identify EBV in breast cancers, most recently Murray and colleagues [97] and Perrigoue and colleagues [107], or to the argument that EBV is confined to lymphocytes located in breast tumors [99].

There may be additional reasons for the differing experimental outcomes. It is apparent that different EBV genes have different influences on different human tissues [102]. For example, the small EBV-related RNAs provide good markers for analyzing EBV associations with some human tumors but are not expressed in many breast tumors. In addition, the expression of EBV genetic material appears to be very low in breast tumors compared with nasopharyngeal cancers or Burkitt's lymphoma. In a very recent study, Arbach and colleagues have demonstrated that EBV genomes are present in approximately 50% of breast cancer specimens, including microdissected cancer cells [100]. The distribution of EBV genomes in tumor cells was variable, even though the morphology of the tumor cells was indistinguishable. This group argue that because EBV is only detected in some breast cancer cells, it is unlikely to be a primary etiological agent.

In addition, EBV is a ubiquitous virus, which infects over 90% of humans. However, infections in developing countries mainly occur in childhood, whereas infections mainly occur in teenagers in economically developed countries. It has been hypothesized that EBV infections in different age groups may result in different cancers, such as Burkitt's lymphoma in Africans, nasopharyngeal carcinoma in people from southeast China and Hodgkin's lymphoma in Western societies [108]. A further reason for the low prevalence of cancers associated with EBV is that EBV mostly acts in concert with co-factors, such as malaria, the consumption of salted fish and possibly with other viruses. Genetic susceptibility of individuals is probably another factor.

Breast tissue from normal women as controls has been used in only four out of the 22 studies shown in Table 3 (in the recent study by Perrigoue and colleagues [107], normal breast tissue from sites adjacent to the tumor were used as normal controls; such tissues are more likely to carry suspect viruses than normal tissue sourced
from normal women). EBV genetic material was rarely identified in normal control breast tissues in contrast to its identification in up to 51% of tumor tissues. While this major difference between cases and controls is strongly suggestive of a role for EBV in breast cancer, the identification by Junker and colleagues of EBV genetic material in nearly half of the milk samples from normal women [103], suggests a need for caution when drawing any conclusions.

The fact that EBV can be transferred by direct contact from lymphocytes to breast epithelial cells suggests that a role in breast carcinogenesis is possible [109]. It can also be argued that as EBV is allegedly not responsive to sex hormones, and as breast carcinogenesis is dependent on sex hormones, EBV should not have a role in breast cancer. However, the conventional wisdom that EBV and related cancers are not influenced by hormones may not be true. In studies conducted during the 1960s on African patients with apparent EBV-associated nasopharyngeal cancers, it was noted that these patients had high urinary estrogen and testosterone excretion levels [110,111]. These observations are compatible with the recent findings from studies in cattle, which show the presence of proteins that activate EBV transcription factors in exocrine and endocrine cells, including such cells in the lactating cow mammary gland [112].

Finally, the correlations between the presence of EBV genetic material in breast tumors and negative estrogen receptor expression is compatible with some role for EBV in breast cancer etiology.

**Additional candidate viruses**

**Bovine leukemia virus**

DNA sequences have been identified in 64% of human breast tumors (n = 166) compared with 19% of normal controls [22]. A total of 74% of women have serum antibodies to BLV [21]. Accordingly, Buehring speculates that BLV could be a rare cause of some human breast cancers.

**Cytomegaloviruses**

These are present in a third of human breast milk samples [113]. However, there is no available evidence that supports an etiological role for cytomegaloviruses (CMV) in human breast cancer.

**Human herpes virus-8 (Kaposi’s sarcoma virus)**

In a recent study of breast cancer in 62 Taiwanese women plus 60 noncancer breast tissue controls, a range of viruses were identified [23]. These included herpes simplex virus-1 (12.9%), EBV (45.2%), CMV (75.8%), HPV (12.9%) and HHV-8 (45.2%) in breast tumors compared with only CMV (66.7%) in normal controls. All the above viral types were present in a breast fibroadenoma control group. This is the first identification of HHV-8 in human breast cancer. HHV-8 is known to act synergistically with HIV to cause Kaposi’s sarcoma [108]. As MMTV is also a retrovirus like HIV, there is therefore a possible role for HHV-8 in breast cancer etiology. There is an excess of secondary Kaposi’s sarcoma in patients with both Hodgkin’s and non-Hodgkin’s lymphoma, leukemia and breast cancer [114], an observation that is in sympathy with the possibility of a range of virus associations between HHV-8, MMTV and EBV.

**Role of viruses as co-carcinogens with other viruses**

It has been shown, both in vitro and in vivo, that retroviruses can be inserted into a herpes virus [115], although this is presumably a very rare event. It is accepted that HIV (a retrovirus) and HHV-8 (Kaposi’s sarcoma virus), can act in concert to cause Kaposi’s sarcoma [108]. As MMTV is a retrovirus and EBV is an HHV, it is possible that some interaction between these viruses may take place during human breast carcinogenesis. There is also experimental evidence that RNAs from genetically distinct retroviruses can exchange genetic information. The speculation follows that MMTV-like exogenous viruses could synergize with human endogenous retroviruses and play some role in human breast cancer. Moreover, it is possible that pseudotyped viruses carrying the genome and capsid of MMTV and an envelope encoded by an endogenous HERV-K may allow the virus enhanced infectability of human cells, or change its target cell spectrum or tropism.

It is also known, as outlined previously, that HPVs acting alone can only immortalize cultured normal human breast epithelial cells and that expression of additional genes are required for full malignant transformation. It is possible that these genes are sourced from other viruses that are present in human breast tissues.

**Discussion, conclusions & future perspective**

The magnitude of dietary influences, fat consumption, genetic and reproductive factors only partly account for the huge differences in breast cancer risk between various populations and
therefore it is likely that there are additional environmental or external factors. As hormone-influenced viruses, which are known to be carcinogenic, have been identified in human and animal breast cancers, but rarely in normal breast tissues, they are logical suspects. In addition, MMTV-like sequences have been identified in 62% of male breast tumors, in which classic female risk factors for breast cancer play little part [116].

In our view, the evidence for a role for HPV in breast cancer carcinogenesis is more substantial than the evidence for MMTV-like viruses and EBV. This is mainly because all eight investigations published since 1999 have consistently identified HPVs in human breast cancers sourced from Europe, the USA, Brazil, China, Japan and Australia (Table 1). Additional evidence is required before any of these candidate viruses, HPV, MMTV-like and EBV can be accepted as being causal of human breast cancer. There is a need to seek evidence of prior infection with these viruses before the development of breast cancer, and additional evidence relating to the means of transmission and some published studies need independent confirmation. This need for independent confirmation of some published studies is important for several reasons. The possibility of contamination is a problem with most studies based on PCR. The historical example of false simian virus-40 data caused by contaminated reagents is a cause for care. The assumption that MMTV could not infect human breast epithelial cells has been shown to be false. The assumption that EBV is not influenced by hormones may not be true.

There is also a need to ensure that the presence of viral genetic material in breast cancer specimens is not parasitic or opportunistic; that is, these candidate viruses do not infect breast tumors after carcinogenesis has occurred. In our view this is unlikely with respect to HPV, MMTV-like viruses and EBV because of the extensive experimental evidence in which MMTV has been shown to cause mouse mammary tumors [12] and the accepted role of EBV and HPVs in other human cancers. It is also plausible that these viruses infect hyperproliferative cells and therefore provide the additional changes required to induce tumorigenesis.

It is also of interest to hypothesize about the overall epidemiology of breast cancer. Why do only some humans get infected with an MMTV-like virus? Why would EBV cause breast cancer in only a small percentage of infected individuals? How does the epidemiology of HPV infection relate to the geographic and ethnic differences in breast cancer rates?

First, we hypothesize that the reason human breast cancer histology and clinical outcomes are so variable (heterogenous) in contrast to MMTV-associated mammary tumors in mice and other rodents, is because of the range of viruses and viral types that may contribute to the etiology of breast cancer.

Second, we hypothesize that because HPV and MMTV are promoted by sex and other hormones, which in turn are influenced by different patterns of nutrition, there will be variations in the incidence of human breast cancer. We further hypothesize that these may be the factors that influence the rapid increase in breast cancer incidence following migration from countries at low risk for breast cancer to those at high risk.

With respect to HPVs, we hypothesize that differences in sexual behavior and differences in HPV types offer a plausible explanation for any ethnic and geographic differences.

Third, we hypothesize that viral types vary in different geographical locations and have varying oncogenicity.

Fourth, we hypothesize that MMTV-like and EBV viruses may influence the immune system through their impact on lymphocytes with individual variations in immune and 'constitutional' responses.

Fifth, we hypothesize that additional viruses to HPV, MMTV and EBV may be involved in human breast cancer. These include HHV-8 (Kaposi's sarcoma virus), BLV, HSV and CMV.

This is a complex set of hypotheses, which will be difficult to investigate and reach definitive conclusions.

Prevention

If conclusive evidence for a role for these viruses in breast carcinogenesis is established, there is a practical possibility of primary prevention. This is because of the successful development of vaccines against infections with HPV-16 and -18. If MMTV is shown to be causal of breast cancer and transmission shown to occur via mother’s milk, primary prevention by vaccines may be impossible because newborn infants will have been exposed to the virus before vaccines can be administered. In addition, the immune response to MMTV exposure may be impaired during the first few weeks.
after birth, as demonstrated experimentally in mice [77]. Primary prevention of EBV infections may become possible with the development of preventive vaccines.

Addendum

A revised fluorescent PCR/laser microdissection-based technique has recently been developed for the specific purpose of identifying MMTV env gene-like sequences in human breast tumours by Zammarchi and colleagues [140]. These sequences have been identified in 33% of human breast cancers. Sequence analysis revealed 96% homology with the MMTV genome. The sequences were not identified in normal breast tissue controls. The techniques provided consistent reproducible data.

This technical development provides confirmation of the positive findings of MMTV-like sequences in human breast cancer but not in normal breast tissues, as discussed in this review.

Acknowledgements

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Executive summary

- There are three striking features of breast cancer. These are: 1) breast cancer is over 100-times more common in women than men; 2) there is an over sixfold difference in incidence and mortality between high- and low-risk populations such as the USA and Japan; and 3) these differences rapidly lessen to equalize within two generations of migration from low- to high-risk countries. Changes in diet and patterns of reproduction after migration partly, but not wholly, explain these phenomena. Hence the emergence of hormone responsive viruses as prime suspects in breast carcinogenesis.

Viral suspects

- Human papilloma viruses (HPVs), mouse mammary tumor virus (MMTV), and Epstein–Barr virus (EBV) are prime candidate viruses for human breast cancer. HPV and MMTV have hormone responsive DNA elements that appear to be associated with enhanced replication of these viruses in the presence of corticosteroid and other hormones. This biological phenomenon is particularly relevant because of the hormone dependence of breast cancer.

Evidence of viral carcinogenesis in human breast cancer

- Viral genetic material for each of these candidate viruses has been identified by PCR in breast tumors but rarely in normal breast tissue controls. Pooled data from controlled studies show substantial odds ratios (ORs) for the presence of viral genetic material in breast tumors compared with normal controls (OR is the frequency of the presence of an agent, such as a virus, in diseased compared with normal tissues).
- The adjusted (for differences in study sizes) OR for the presence of HPV gene sequences in breast cancer compared with normal controls is 7.18 (confidence interval [CI]: 1.82–28.47). The adjusted OR for the presence of MMTV gene sequences in breast cancer compared with normal controls is 27.55 (CI: 12.26–61.91).
- Histological characteristics of HPV-positive human breast tumors are similar to HPV-positive human cervical cancer. Histological characteristics of some MMTV-positive human breast tumors are similar to MMTV-induced mouse mammary tumors. MMTV-like DNA sequences have been detected in breast cancer epithelial cells but not in normal breast epithelial cells.
- Normal breast cell cultures are transformed by exposure to HPV. MMTV integrates randomly in the genome of infected cells and can activate cellular oncogenes, initiating a pathway of transformation. In addition, it has been shown that MMTV can have a direct effect upon the phenotype of three-dimensional cultures of normal human breast cells. Normal breast epithelial cells are immortalized by EBV.

Conclusion

- These and additional data provide substantial, but not conclusive, evidence that HPV, MMTV and EBV may have a role in the etiology of human breast cancer. If conclusive evidence for a role of these viruses in breast carcinogenesis can be developed, there is a practical possibility of primary prevention of some human breast cancers, for instance using the recently developed anti-HPV vaccines.

Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.


25. Latest comprehensive review of risk factors for breast cancer; however, the possible role of viruses does not rate a mention!


30. MacMahon pioneered many of the original studies on breast cancer risk factors.


39. Dimitrios Trichopoulos has originated many of the etiological hypotheses for breast cancer.


41. De Villiers E-M, Sandstrom RE, zur Hausen H, Buck CE: Presence of papillomaviruses...

- First study to demonstrate that HPV in breast cancer are associated with typical histology.


- First report of a typical human breast cancer histopathology associated with mouse mammary tumor virus (MMTV)-like virus.


- Provides sound experimental evidence of free floating HPV viruses leaking from epithelial cells.


- Study over-riding the conventional wisdom that MMTV cannot infect human epithelial cells.


- Clear demonstration that MMTV-like sequences are located in breast cancer cells but rarely detected in normal breast epithelial cells.


PERSPECTIVE – Lawson, Günzburg & Whitaker

100. Lawton, Günzburg & Whitaker: First demonstration that MMTV can transform normal breast epithelial cells in culture. Katz and colleagues also show for the first time that an MMTV oncogene may be involved in human breast carcinogenesis.
Viruses and human breast cancer — PERSPECTIVE


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