Commentary

From Bittner to Barr: a viral, diet and hormone breast cancer aetiology hypothesis

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Abstract

It is hypothesized that the human homologue of the mouse mammary tumour virus (HHMMTV) and other viruses, such as human papillomavirus (HPV) and Epstein–Barr virus (EBV), act as cofactors with diet, oestrogens and other hormones in the initiation and promotion of some types of breast cancer in genetically susceptible women. It is further hypothesized that diet influences the risk of breast cancer, through its influence on oestrogen metabolism and that of other hormones, in combination with genetic and infectious agents.

Keywords: breast cancer, diet, Epstein–Barr virus, human papillomavirus, mouse mammary tumour virus, oestrogens

Introduction

In 1943, Bittner [1] demonstrated three cofactors in the development of spontaneous mammary tumours in mice. These were inherited susceptibility, hormonal influences, and a transmissible influence in mother's milk. This transmissible influence has since been shown to be a retrovirus, now known as the mouse mammary tumor virus (MMTV). This virus is oncogenic in the oestrogenic milieu of female mice of strains with a genetic susceptibility to mammary tumors [2]. Over the years, considerable indirect and limited direct evidence has emerged that suggests that an almost identical retrovirus to the MMTV, plus additional cofactors, may influence human breast carcinogenesis. This virus has become commonly known as HHMMTV. In addition, evidence has recently emerged that suggests additional viruses, such as the HPV and EBV, may also initiate or promote breast carcinogenesis [3–7].

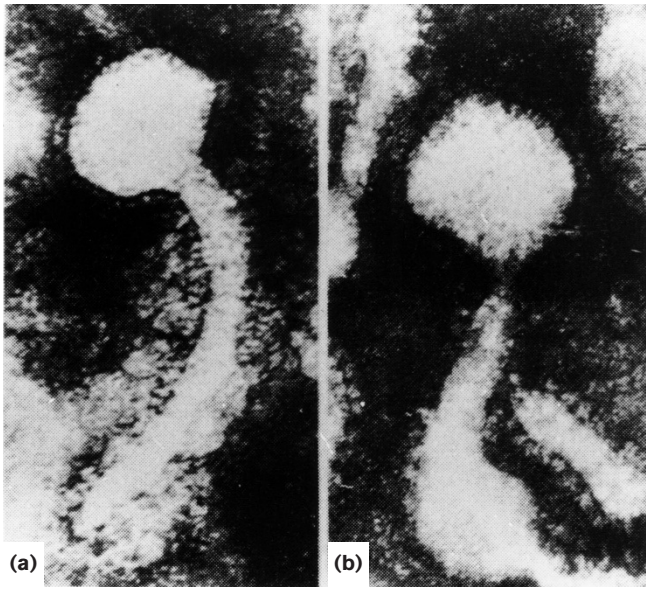
HHMMTV and MMTV

In 1971, Moore *et al* [2] demonstrated that human milk from women who are at high risk for breast cancer contains particles that are morphologically similar to the MMTV. As shown in Fig. 1, these particles have a 'mushroom' shape, with spikes on the viral envelope. Schlom *et al* [8] showed that such particles had reverse transcriptase activity, which is found in oncogenic retroviruses. Evidence has since accumulated showing the presence of a retrovirus that is homologous to MMTV in human breast cancer tissues [9,10], in cultures of normal human breast cells [11] and in cultures of human breast cancer cells [12,13].

In 1971, Charney and Moore [14] showed that the serum of humans with breast cancer decreased the virility of MMTV, which suggested that antibodies to proteins of the

EBV = Epstein–Barr virus; HHMMTV = human homologue of the mouse mammary tumour virus; HPV = human papillomavirus; MMTV = mouse mammary tumour virus.

Figure 1



Electron microscopy images of human and mouse mammary viruses taken in 1971. **(a)** Electron microscope image (×180,000) of particles from human milk. These particles are almost certainly the virions of the HHMMTV. **(b)** Image (×180,000) of particles from mouse milk. These particles are of the MMTV. The blurred edges of the particle images appear to be due to protein surface projections on the membrane of the viruses. The morphological characteristics of the particles in both the human and mouse milks appear to be almost identical and are unique among all viruses. When these characteristics are considered in conjunction with the 98% homology of the nucleotide sequences between the HHMMTV and MMTV, it appears likely that these viruses are variants of each other. Reprinted by permission from *Nature* [2] copyright 1971, Macmillan Magazines Ltd

virus may be present in humans. Antibodies that are reactive with MMTV are present at much higher levels in serum of humans with breast cancer than in serum of healthy women [15]. Such antibody levels in women with breast cancer vary from below 5% (in Chinese) to over 60% (in East Africans) among different populations [15]. In addition, Stewart *et al* [16] collated data that suggest that the geographic variation in human breast cancer parallels the presence of the wild, but common house mouse, which may have acted as an animal host of the MMTV.

Gene sequences that are congruent with those of MMTV were detected and cloned in human breast cancer tissues by Callahan *et al* in 1982 [17]. Such detection had been difficult because of the presence of endogenous retroviral sequences within the human genome that are homologous with MMTV sequences. This problem has been largely resolved by Wang *et al* [18] by using sequences that are homologous to MMTV sequences, with low homology to human endogenous retroviruses. MMTV *env* gene sequences were found in 30–40% of breast cancer tissues in women from various populations. The *INT6* gene is a common integration site for MMTV in mouse mammary tumours, and a human homologue with identical *INT6* peptide has been identified in human breast cancer tissues [19].

Gene products of MMTV are present in concentrations that are 1000–10,000 times greater in malignant than in normal cells of mice [20]. It is not known whether this phenomenon occurs with respect to HHMMTV, but it may explain the difficulty experienced by Wang *et al* [18] in detecting the HHMMTV in normal breast epithelial cells. Endogenous HHMMTV has since been detected in normal breast epithelial cells by Soble *et al* [21] and our group (Rawlinson *et al*, unpublished data). The MMTV is highly mammotrophic, but it can cause lymphomas in mice [20]. The HHMMTV also appears to be mammotrophic [18]. However, HHMMTV has recently been identified in both breast and lymphoma tissues in several women with simultaneous diagnosis of breast cancer and non-Hodgkins lymphoma [22]. Non-Hodgkins lymphoma occurs more frequently in women with breast cancer than in the general population [23]. *In vitro* studies of human breast cancer cell lines [24] have shown that administration of oestrogen followed by progesterone stimulates the expression of human endogenous retroviruses in the genome.

The MMTV may be exogenous or endogenous [25]. The exogenous MMTV particles are transmitted in maternal milk but are not infectious for other mice [25]. The endogenous noninfectious MMTV particles are transmitted as part of the germline DNA [25]. Although both exogenous and endogenous forms of the virus appear able to induce mammary carcinogenesis independently in some mouse strains, they may also combine and be carcinogenic in the breast [25]. This phenomenon of recombination of exogenous and endogenous retroviruses, which then induce cell proliferation, is known in other viruses [26]. Therefore, it is possible that recombination of exogenous and endogenous HHMMTV may occur, potentially resulting in enhanced carcinogenesis.

Hormone and MMTV synergy

There is experimental evidence that glucocorticoids, insulin, epidermal growth factors, oestrogens and progestins synergize with MMTV in genetically susceptible female mice to cause mammary cancers [20]. Male mice are also exposed to exogenous and endogenous MMTV. Although endogenous MMTV has been isolated in the testes of male mice, these mice very rarely develop mammary cancers [25]. One possibility is that circulating oestrogen levels in male mice are not sufficiently high to promote MMTV activity. However, an antigen that is related to MMTV has been identified in approximately 90% of human male mammary carcinomas [27].

It is possible that MMTV is the initiator, and oestrogens and other hormones are the promoters of some mammary cancers in mouse models. However, the presence of MMTV is not obligatory for all mouse mammary cancers [20].

Are there other viruses that cause breast cancer in humans?

It has been shown [3] that HPV can immortalize normal human mammary epithelial cells. This leads to the possibility that HPV may be associated with breast as well as cervical carcinogenesis. In 1992, Lonardo *et al* [4] demonstrated the presence of HPV type 16 in 29% of breast tumors and metastatic lymph nodes using polymerase chain reaction techniques. Recently, HPV-16 has been identified in both breast and cervical cancer tissues in Norwegian women with concurrent breast and cervical cancer [5]. In addition, HPV-33 has been identified in breast tumors of 41% of Chinese and 11% of Japanese women with breast cancer [6].

Bonnet *et al* [7] identified EBV in breast cancer tissues in French women. This finding has provoked controversy, because it has been suggested that the EBV identified by Bonnet *et al* may have been present in infected lymphocytes in the breast [28]. EBV is ubiquitous in the population and is present in many tissues. It is the initiator of Burkitt's lymphoma, and it is highly associated with some cancers of epithelial origin such as nasopharangeal carcinoma. Although speculative, it is possible that EBV may enhance the action of HHMMTV, because it is known that some viruses remain dormant unless activity is promoted by other viruses [29,30].

Diets, oestrogens and breast cancer

Endogenous oestrogens are central to the aetiology of breast cancer [31]; in the absence of oestrogens, breast cancer does not occur. A recent prospective study of Japanese women [32] indicated that levels of serum oestrogens are positively correlated with risk of breast cancer, with a greater than threefold odds ratio in women with the highest as compared with the lowest serum oestradiol.

In humans there are strong associations between dietary pattern and level of circulating oestrogens, with energy-rich diets correlated with high circulating oestrogens [33]. However, evidence from huge prospective studies [34] has strongly suggested that there is no association between dietary fats and breast cancer in humans. On the other hand, the individuals studied were all from Europe and North America, where the consumption of fats and energy far exceed those of populations with low risk of breast cancer. Well-conducted case–control and ecological studies in populations with low risk of breast cancer, such as Chinese, Japanese and Indonesian populations, have shown that the risk of breast cancer is up to seven times higher in women who consume the highest levels of fats and energy in those populations [35–37]. These findings parallel the consistent correlations between *per capita* fat and energy consumption and breast cancer risk between countries [38]. In addition, there is emerging evidence from studies in humans [39] that different types of dietary fat may have different influences on breast carcinogenesis. N-6 polyunsaturated fats (as found in vegetable margarines) may increase risk, and n-3 polyunsaturated fats may reduce risk.

The associations between consumption of fats and energy and mammary carcinogenesis in experimental rodents is well established [40]. Experimental evidence in mice has shown that diets that are high in n-6 polyunsaturated fats (mainly sourced from corn) are associated with high oestrogen receptor expression in mammary epithelial cells and increased incidence of mammary tumours [41]. In addition, such diets have been shown [42] to accelerate the transcription of endogenous MMTV and to accelerate carcinogenesis.

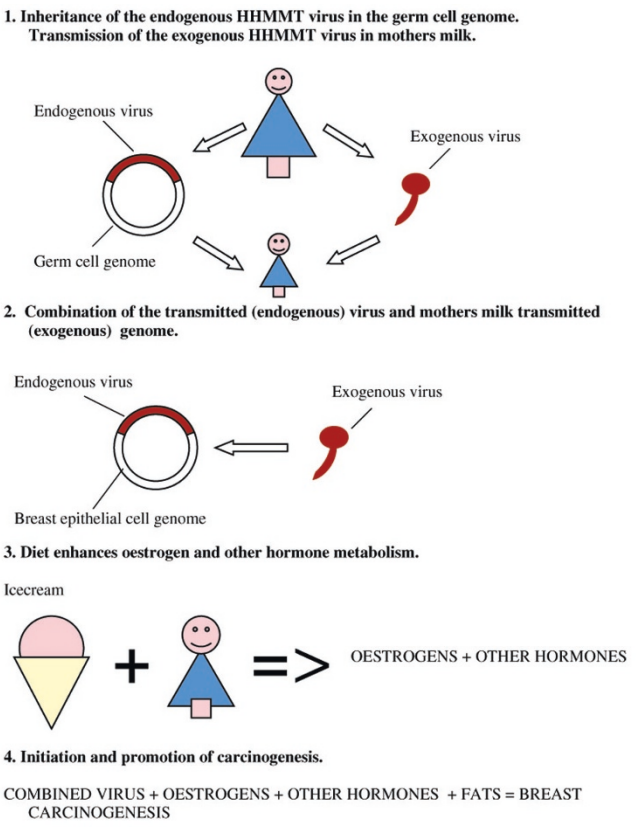
Epidemiology

The epidemiology of breast cancer is well established. The most striking features of human breast cancer are the 100-fold greater incidence in females than in males, and up to 10-fold greater incidence of breast cancer in Western as compared with some Asian countries [31]. There is increased risk of breast cancer associated with early age of menarche, late age of menopause, late age of first full-term pregnancy, alcohol consumption and increased postmenopausal weight [31]. It is also possible that some forms of breast cancer originate during foetal life [31]. With the exception of genetic influences, these epidemiological features of breast cancer are probably all associated directly or indirectly with oestrogen physiology, which in turn may be associated with maternal diets during gestation and lifetime diets [31]. The viral/diet/hormone hypothesis is compatible with these epidemiological features, particularly as endogenous retroviruses may remain dormant for decades.

The case against a viral aetiology of breast cancer

There is seemingly strong evidence against a infectious cause of some types of breast cancer that is transmitted by mothers milk. Nearly 30 years ago, Fraumeni and Miller [43] summarized this evidence. Breast cancer rates are low in countries where breast-feeding is prolonged, and rates are increasing in countries where breast-feeding is declining; breast cancer occurs equally in maternal and paternal lines; and mother and daughter occurrences of breast cancer are not associated with breast-feeding. In addition, in a recent, large, case–control study of over 5685 US women with breast cancer [44], no evidence was found that having been breast-fed increased breast cancer risk in either premenopausal or postmenopausal

Figure 2



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women. An implication of this study is that human mothers' milk does not transmit an infectious agent. However, it was also observed in that study that women with breast cancer had a strong familial history of breast cancer, an observation that is compatible with an endogenous virus transmitted with germline cells, which may influence carcinogenesis in genetically susceptible individuals.

Conclusion

When considered as a whole and as shown on Fig. 2, this evidence suggests that HHMMTV, and possibly other transmissable viruses, in association with diet, steroid and other hormones and genetic susceptibility, has a role in human breast carcinogenesis. It is possible that HHMMTV is transmitted as particles (virions) in mother's milk and as part of the endogenous viral genome in the germline. Given the precedence of the association between MMTV and murine cancer, it is also possible that the exogenous HHMMTV (as virions in human maternal milk) may combine with endogenous HHMMTV, which then has a carcinogenic influence. Given the strong evidence against a human milk-borne infectious agent, it may be that viral human breast carcinogenesis only occurs if the endogenous virus is present in the genome of the breast epithelial cells, in addition to the exogenous virus that may be present in breast milk. Diets with high intakes of energy may lead to increased levels of oestrogen and other hormones that may enhance expression of HHMMTV.

Many of these possibilities were suggested nearly 30 years ago [45]. At that time there was concern that, if there was a human breast cancer virus that had the milk-transmitted and inherited characteristics of the MMTV, then primary prevention would probably be useless in the face of immunological tolerance. However, if the above hypotheses are shown to be true, then there is a possibility of primary prevention by dietary intervention aimed at a reduction in serum oestrogens, vaccine immunization for viral infections and the use of hormone-modifying agents such as tamoxifen for women with HHMMTV [46].

All of these hypotheses are testable.

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