**Recent developments linking retroviruses to human breast cancer: infectious agent, enemy within or both?**

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Evidence is accumulating that one or more beta-retrovirus is associated with human breast cancer. Retroviruses can exist as an infectious (exogenous) virus or as a part of the genetic information of cells due to germline integration (endogenous). An exogenous virus with a genome that is highly homologous to mouse mammary tumour virus is gaining acceptance as possibly being associated with human breast cancer, and recently furnished evidence is discussed in this article, as is the evidence for involvement of an endogenous human beta-retrovirus, HERV-K. Modes of interaction are also reviewed and linkages to the APOBEC3 family are suggested.

**Background**

It has long been speculated that retroviruses might play a role in human breast cancer. Initial interest focused on a potential role for mouse mammary tumour virus (MMTV) and a quite comprehensive review on the early evidence for this appeared in Nature as early as 1971 (Moore et al., 1971). This and later papers published described the detection of virus particles, virus proteins and antibodies that react with MMTV in the serum of patients, and the presence of MMTV-related nucleic acids in breast cancers. However, in the 1980s, MMTV-related endogenous sequences (HERVs) were discovered and they seemed to provide an explanation for most of the evidence that had amassed implicating MMTV in human breast cancer (Lawson et al., 2006). Moreover, this discovery spawned a new hypothesis that HERVs, and in particular HERV-K, play a potential role in the development of breast cancer. This view was challenged in the mid-1990s, when the work of the group of Beatriz Pogo (Wang et al., 1995), quickly followed by others, showed that MMTV or a virus like it might be a cause of at least some cases of breast cancer in humans (reviewed by Lawson et al., 2006). The Pogo group alone has identified the presence of an MMTV proviral structure in human breast cancer cells (Liu et al., 2001), and shown the presence of virus particles produced by primary human breast cancer cells (Melana et al., 2007) and of virus proteins in human breast cancer cells (Melana et al., 2010). Importantaly, many of these findings have been confirmed and extended by other groups (reviewed by Lawson et al., 2006), albeit not by all researchers (reviewed by Joshi & Buehring, 2012).

**Human breast cancer and MMTV**

The heightened interest in MMTV as a human breast cancer virus is reflected by the fact that in the last year alone there have been a number of reviews on the key evidence that MMTV, or a highly related virus, is present in a proportion of human breast cancers (Mason et al., 2011; Alibek et al., 2013; Salmons & Günzburg, 2013). Naushad and colleagues have also recently published a primary analysis of the prevalence of MMTV in the Pakistani population. They amplified a 666 bp MMTV envelope and a 630 bp LTR sequence from tissue biopsies and peripheral blood from breast cancer patient samples and detected MMTV-like virus env and LTR DNA sequences in 20 and 26% of breast tumour samples, respectively, from a total of 80. In this study, no significant association was observed between age, grade of disease or lymph node involvement and the prevalence of MMTV-like sequences (Naushad et al., 2014). A second recently published comprehensive meta-analysis in which 22 previous studies were analysed with respect to the association between MMTV infection as detected by PCR-based techniques and breast cancer concluded that MMTV infection significantly increases the risk of breast cancer, by about 15-fold. However, even though the data included in this study had to fulfil a number of well-defined criteria, the meta-analysis was limited by substantial heterogeneity across studies due to differences in cell selection methods and/or detection methods used. Nevertheless, the prevalence of MMTV in breast cancer tissue was much higher in Western patients than in Asians, in line with what had previously been concluded (Wang et al., 2014).
Excitingly, a recent publication by Pogo and colleagues has recently provided new evidence that a virus highly related to MMTV might be involved in human breast cancer (Nartey et al., 2014). This paper reports the presence of the virus [named by the authors ‘human mammary tumour virus’ (HMTV)] in milk from lactating women. In this study, milk from two groups of woman was compared: (1) one who had undergone breast biopsies on suspicion of breast cancer at some point in their lives or were scheduled for breast biopsy on the basis of either clinical or radiological findings (high risk of breast cancer); and (2) one without biopsies as a control group. The major finding of the study was that although MMTV-related sequences could be found in both groups, the high-risk group that had undergone or were about to undergo biopsy showed a threefold higher positivity for the virus.

These three recent studies support the notion that an MMTV-like virus may be involved in at least some cases of human breast cancer and also go some way towards answering some of the issues raised by other commentators (Lawson et al., 2006; Mason et al., 2011; Salmons & Günzburg, 2013).

The Pogo group study was not the first to demonstrate the presence of MMTV in human milk (Johal et al., 2011) and there is thus some comfort from the fact that two independent groups have shown this. Nevertheless, there are some issues and caveats. The percentage of patients showing MMTV-like sequences (20.55 %) in the milk (Nartey et al., 2014) is somewhat lower than the 38–40 % previously reported by that and other groups (Lawson et al., 2006). This may be due to the fact that the study group was a high-risk group rather than to women who actually had breast cancer, in which the incidence was 38–40 %. The virus appears also to have been detected in a crude pellet in this study (Nartey et al., 2014) and thus formally may not have been virus, but rather DNA/RNA associated with particulate matter. Ideally, virions should have been isolated by isopycnic banding to strengthen the contention that the PCR signal came from a virus. Admittedly, if too little virus was present this could have been an issue; however, it should be noted that MMTV-infected mouse milk contains enough particles to allow this (Salmons & Günzburg, 1987; Ross, 2010). We recently discussed the issue that, even though MMTV sequences have been detected in human breast cancer tissue, highly sensitive detection methods often have to be used, implying that relatively low levels of virus are present as compared with those in mice (Salmons & Günzburg, 2013). This has implications for the mechanisms of tumorigenesis: although it is quite clear that MMTV influences mouse cellular genes by enhancer insertion and that a number of these genes activated in the mouse are activated in human breast cancer (Callahan et al., 2012), including wnt-1 (Lawson et al., 2010), the likelihood of this happening in humans is linked to the number of infection events with a randomly integrating virus like MMTV (Faschinger et al., 2008), which suggests that the likelihood of a relatively low-titre virus integrating near one of the genes associated with breast cancer is quite low. Other putative mechanisms of virus-mediated tumorigenesis have recently been discussed (Salmons & Günzburg, 2013).

Another issue is that all of the studies to date have looked at the presence of MMTV env (or LTR) sequences because this region shows most sequence divergence from HERVs, but this would strengthen the contention that a virus is present if the authors had also looked for gag and/or pol sequences, although presumably they can still do this. Worryingly, in the study of Nartey and colleagues, mouse DNA was found to be present in one of the DNA samples and, while all the other samples were shown to be negative for mouse DNA sequences (Nartey et al., 2014), this is a potential reason for concern. Another potential inconsistency is that that study suggests a hormonal linkage between HMTV (MMTV) infection and breast cancer (Nartey et al., 2014), in line with their previous findings in gestational breast cancer (Wang et al., 2003). However, the metadata analysis reported by Wang and colleagues which includes these data concludes a lack of positive association between MMTV infection and expression of ER, PR, HER2, p53 or histological grades (Wang et al., 2014), so further independent studies may be required to clarify this.

Human cancer and HERVs

As mentioned above, there appear to be two camps advocating a role for a human retrovirus in breast cancer—one endorsing endogenous HERVs as perpetrators and the other exogenous MMTV. A number of groups have provided evidence to support a role for HERV in breast cancer (for recent reviews see Cegolon et al., 2013; Downey et al., 2014). Although a recent study examining the prevalence of integrated forms of two common HERV-K proviruses failed to find a significant difference between individuals diagnosed with breast cancer and those without history of the disease, the authors point out that their findings do not rule out the possibility that rarer HERV-K proviruses are involved in a subset of breast cancers or will provide a meaningful biomarker of this disease (Wildschutte et al., 2014). Indeed, most recently, antibodies against HERV-K and HERV-K-specific mRNA have been proposed as serum biomarkers of early stage breast cancer (Wang-Johanning et al., 2014).

The involvement of an MMTV-like virus or HERV-K in human breast cancer is not necessarily an ‘either/or’ situation (i.e. they are not mutually exclusive). Even in the mouse, it is documented that both endogenous and exogenous viruses individually can cause mammary tumours (Salmons & Günzburg, 1987; Ross, 2010), but perhaps crucially they can also jointly play a role in tumorigenesis. This cooperation can be in the form of direct interaction, for example in GR mice. Mammary tumours in GR mice have been linked to the endogenous provirus Mtv-2, which produces an exogenous virus, but also the endogenous provirus Mtv-17 produces transcripts...
that can be packaged into virions, shed into milk and contribute to recombinant proviruses. These recombinant proviruses essentially consist of an Mtv-2 sequence with the envelope sequences from Mtv-17, and such recombinant proviruses are found in a small percentage of virus-induced mammary tumours (Golovkina et al., 1996). In this respect, it is of interest that the integration pattern of a resurrected HERV-K, in which various fragments of defective HERV-K virus genomes were linked together to produce an infectious provirus, was shown to resemble that of MMTV (Faschinger et al., 2008; Brady et al., 2009).

Additionally, an indirect role of endogenous MMTV proviruses, in which super-antigens encoded by the endogenous proviruses contribute to tumorigenesis by shaping the host immune system, has been documented (reviewed by Holt et al., 2013). Although MMTV-specific super-antigen primers are able to amplify sequences from the human genome (Indraccolo et al., 1995), to date there is little evidence that HERVs encode super-antigens.

As well as recombinant viruses, there is also the potential for the production of pseudotyped viruses. Heidmann’s group showed some years ago that the envelope of HERV-K108 is functional in that it can pseudotype simian immunodeficiency virus and confer infectivity (Dewannieux et al., 2005). Moreover, a resurrected, consensus HERV envelope has been shown capable of forming pseudotypes with human immunodeficiency virus (HIV) (Lee & Bieniasz, 2007). It remains to be determined whether pseudotypes consisting of virion components from HERV and MMTV can be produced in cell culture, let alone in vivo.

Retroviruses can interact with each other and modify disease induction. HERV-K activation and co-expression with HIV-1 has been observed during HIV-1 infection. Monde and colleagues found that the release efficiency of HIV-1 Gag was reduced by threefold when the HERV-K Gag was co-expressed and that HERV-K Gag co-localized with HIV-1 Gag at the plasma membrane and co-assembled in virus particles, leading to a similar reduction in HIV-1 infectivity (Monde et al., 2012). These findings raise the possibility that both exogenous and endogenous viruses may cooperate to cause human breast cancer and would help explain the differences between the classical mechanisms of tumour induction that have been described for MMTV in the mouse. Some potential mechanisms of tumorigenesis have been discussed previously (Salmons & Günzburg, 2013), although only in the context of an MMTV-like virus playing a role.

A central role for APOBEC3 in breast and other cancers

Cellular enzymes of the apolipoprotein B editing complex (APOBEC) family encode deaminase enzymes that edit DNA and/or RNA sequences and play a central role in the control of virus infections, including retroviruses. APOBEC enzymes normally function in innate immune responses, including those that target retroviruses, suggesting links between mutagenesis, immunity and viral infection in the process of cancer development (Henderson et al., 2014). Many viruses have evolved mechanisms to counteract APOBEC effects, and thus drugs enhancing APOBEC activity are being developed. Interestingly, inactivating mutations and deletions in APOBEC3 seem to play a possible role in breast cancer development. This could lead to activation or enhanced expression of exogenous and endogenous retroviruses, as proposed by Downey and colleagues (Downey et al., 2014). Aberrant expression of APOBEC3 has also been shown to be induced by DNA viruses such as human papilloma virus, specifically in breast cancer (Ohba et al., 2014), and also a deletion polymorphism involving the APOBEC3 gene cluster on chromosome 22 is associated with elevated risk of breast cancer (Long et al., 2013; Nik-Zainal et al., 2014). Moreover, a specific APOBEC mutation pattern has been described in a number of cancers, including breast cancer, in particular the HER2-enriched subtype of breast cancers, suggesting that this is functionally linked with cancer development (Roberts et al., 2013). This mutation pattern has been shown to be linked to APOBEC3B expression specifically in breast cancer and leads to DNA damage that could select TP53 inactivation, thereby resulting in tumour heterogeneity (Burns et al., 2013). Taken together, all of these studies suggest that the APOBEC family and particularly APOBEC3 may play a central role in the early stages of breast cancer induction (Lawson, 2014). However, although an intriguing possibility, additional studies are required to formally demonstrate a link between MMTV and/or HERV-K and APOBEC family members and breast cancer.

Next-generation information on retroviruses and breast cancer

The advent of next-generation sequencing offers a much more comprehensive means to study retroviral integration sites and has been employed, for example, by Marchi and colleagues to analyse potential integration sites in the DNA of 358 individuals that may not be present in the human reference genome sequence (Marchi et al., 2014). Interestingly, they found 17 novel HERV-K integration loci using this technique.

Similarly, Shingo Miyauchi, working in Sydney, Australia, has preliminary data based on next-generation sequencing (S. Miyauchi, unpublished), which confirm the presence of MMTV env sequences in breast cancers. While these findings must be verified, the data are supportive of the PCR-based findings that MMTV env sequences are present in breast cancers that have developed in women from many countries (Wang et al., 2014).

Conclusion

Slowly evidence is amassing that a retrovirus might be involved in certain cases of human breast cancer, but there is still a long way to go. Many groups have found evidence...
for the potential involvement in human breast cancer of a virus highly homologous to MMTV, a well-known aetiological agent of mammary cancer in mice. Recent data have confirmed and extended support for a role of MMTV (or its human homologue, HMTV), but HERV-K as well as other viruses could also play a role either individually or in concert, perhaps via APOBEC enzymes. The lack of many of the hallmarks of MMTV-induced tumours supports the notion of both viruses acting in concert (assuming that a virus is indeed involved). The next few years may finally see general acceptance that retroviruses play a seminal role in the development of breast cancer as well as the development of diagnostic tools and potential antiviral therapies.

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References


