An alternative approach to medical genetics based on modern evolutionary biology. Part 4: HERVs in cancer

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DECLARATIONS

Competing interests
None declared

Funding
None

Ethical approval
Not applicable

Guarantor
FPR

Contributorship
FPR is the sole contributor

Acknowledgements
The author would like to thank Massimo Palmarini, Klaus Roemer, Corrado Spadafora and Oliver Quarrell for their assistance with this paper

Introduction

In Part 1 we saw that cancer is a multistep process involving complex genetic abnormalities that deregulate signalling pathways, and it involves the cooperation of multiple deregulating genetic pathways.[1] Given the widespread involvement of HERVs and related products in human genetic chemistry it is likely that they will be involved in carcinogenesis. We might anticipate that such viral involvement will arise from the known oncogenic potential of viruses, particularly retroviruses. We also need to consider that HERVs, unlike exogenous retroviruses, have been subject to selection working at holobiontic level within the human genome over long time periods. From this we might anticipate additional potential for carcinogenesis deriving from the cooption, or dysregulation, of established symbiotic roles of whole viruses, viral genes and viral regulatory sequences involved in normal genetic pathways.

Viruses and cancer

Approximately 20% of human cancers have been attributed to virus infection, and, in the opinion of virologist, Robin A Weiss, other cancers may also have a viral component.[2] In 2008 the German pathologist, Harald zur Hausen, was awarded the Nobel Prize for Medicine for his discovery that the human papilloma virus (HPV) is the cause of 99% of cancers of the cervix, and a majority of vulval, vaginal and penile cancers – leading to the current vaccination programme.[3–5] Other examples include the hepatitis B and C viruses, which cause hepatocellular carcinoma,[6] and the Epstein-Barr virus, which causes Burkitt's lymphoma, and may also be linked to nasopharyngeal carcinoma and some of the lymphomas that complicate AIDS.[7] Many such viruses are DNA-based, and contain genes that are directly oncogenic through insertion into the host DNA. For example, HPV causes cervical cancer through two viral proteins, E6 and E7, which interfere with the regulation of normal cell division by two key human proteins, Rb and p53.[8]

Exogenous retroviruses are carcinogenic throughout the animal kingdom, including marine invertebrates, birds, marsupials and a wide variety of placental mammals.[9,10] These RNA-based retroviruses are usually oncogenic through common indirect pathways, such as integration of the virus adjacent to a cellular oncogene, or incorporation of a host oncogene within the retroviral genome, or through more complex interactions involving viral LTRs and host regulatory pathways, such as tumour suppression genes.[11,12] HIV-1 also involves virus-specific oncogenic pathways. For example, the various non-Hodgkin's lymphomas associated with AIDS may involve activation of the oncogene *c-MYC*, inactivation of p53, and co-infection with the Epstein-Barr virus.[13] With effective modern treatment, the once-frequent Kaposi's sarcoma is now a rarity, confirming the importance of AIDS-related immunosuppression in the genesis of tumour progression.[14] HTLV-1 also induces oncogenesis through a virus-specific regulatory protein, called Tax, although the precise oncological pathway is still under evaluation.[15] Genetic screening for exogenous retroviral oncogenesis in experimental mice has revealed more than a hundred loci with carcinogenic potential, including the involvement of established human oncogenes.[16–18] This suggested that HERVs might also possess significant oncogenic potential.

HERVs in carcinogenesis

Endogenous retroviral particles were first reported in platelets from patients with myeloproliferative

disorders in 1975.[19] Subsequently, reverse transcriptase activity coupled with EM pictures of HERVs have been detected in platelets from patients with primary proliferative polycythaemia and essential thrombocythaemia,[20] HERV-K protein synthesis (*gag* gene) has been detected in megakaryocytes from stem cells cultured from the peripheral blood of patients with essential thrombocythaemia,[21] with packaging of the *gag* protein into HERV-K viruses budding from the cell membrane of the megakaryocytes. While this suggested that HERV-Ks might be implicated in the myeloproliferative disorders, no firm conclusions could be drawn as to whether the HERVs were causative or acting in a responsive role.

HERV-H has been found in leukaemia and various cancer cell lines as well as cancers of the lung, stomach, intestine, bone marrow, bladder, prostate and cervix,[22–25] HERV-K with melanoma, seminomas, the blood of leukaemia patients, teratocarcinomas and breast cancer lines,[26–34] and HERV-E in prostate carcinoma.[35] Other researchers have linked HERV-related sequences, such as LINE-1s, SINES and *Alus* to a variety of cancers,[36] including oesophageal adenocarcinoma.[37] It is too early to confirm any putative role of HERVs and products in such associations, but one study has reported a possible mechanism of HERV-induced malignancy. A relatively rare pattern of stem-cell myeloproliferative disorder has been linked to translocations on chromosome 8 in a region involving the *FGFR1* gene, which encodes one of the tyrosine kinase receptors for fibroblast growth factors. The resulting syndrome is characterized by myeloid hyperplasia, frequent peripheral blood eosinophilia and B- or T-cell lymphoblastic leukaemia or lymphoma.[38,39] Guasch and colleagues have reported the fusion of a HERV-K element sequence with *FGFR1* sequences at the break point on chromosome 8 in one out of eight 'partner gene' examples of this disorder, with subsequent translocation to chromosome 19 in a patient suffering from an atypical myeloproliferative syndrome.[40] This might imply non-allelic recombination between HERV elements on the two chromosomes.

In a series of papers, Schulte *et al*. have described how the insertion of a HERV into the intron sequence immediately upstream of the first coding exon of the human growth factor gene, pleiotrophin (PTN), generated an additional promoter with trophoblast-specific activity.[41] Further studies of the HERV suggested that it derived from the recombination of a HERV-E and RTVL-1, generating a novel viral element containing one *gag*, two *pols* and two *env* domains, flanked by LTRs. Since it retained the defining primer binding of a HERV-E, the authors classed it as a novel HERV-E.[42] PTN stimulates growth and transformation in fibroblasts and epithelial cells, and it plays an important role in the developments of human melanoma and human trophoblast-derived choriocarcinoma. The authors also demonstrated transcription of messenger RNA for the fused HERV-E.PTN domain in normal human trophoblast cell cultures as early as 9 weeks after gestation as well as in full-term placentae. The fused domain was not present in mice, or rhesus monkeys, but was common to humans, chimpanzees and gorillas, confirming a holobiontic evolutionary event dating to about 25 million years ago that had resulted in a new PTN promoter. Transcription of the fused domain was also a feature of choriocarcinoma cell lines but not tumour cell lines derived from the embryoblast (teratocarcinomas) or other lineages. This suggests that the choriocarcinoma might be co-opting a symbiotic HERV function involved in the proliferative and invasive behaviour of normal trophoblasts during placentation. Deletion of the retroviral-derived section of the promoter sequence prevented the growth, invasion and angiogenesis that would normally accompany the tumour development.[43]

In an elegant body of work over the last decade, Roemer, Armbruester and colleagues have presented a growing litany of evidence for the fact that HERV-K viruses may play significant roles in carcinogenesis. They first noticed the association between high titres of antibodies to HERV *gag* and *env* and germ cell tumours, such as seminomas and teratocarcinomas.[44,45] HERV-K viruses possess a virus-specific gene known as *rec*, or cORF, which is the functional homologue of the HIV-1 gene, REV. This codes for proteins that enable daughter virus production after infection. The same authors have shown that HERV-K *rec* expression interferes with germ cell development in transgenic mice, where it may also cause carcinoma *in situ*.[46] In particular they have identified a novel gene, *Np9*, within the HERV-K *env* genetic domain that gives rise to a protein localized predominantly in the cell nucleus. The expression of *Np9* in various tumours suggest that it may be playing a role in carcinogenesis,[47,48] and this

in turn may be mediated through interactions between HERV proteins Np9 and Rec with the promyelocytic leukaemia zinc finger protein, a transcriptional repressor and chromatin remodeller that has been implicated in cancer and the self-renewal of spermatogonial stem cells.[49,50] However, even this well-researched line of evidence, while increasingly suggestive of a carcinogenic role for certain HERV-K genes, does not yet amount to conclusive evidence.

Members of the HTDV/HERV-K family can express themselves as viral particles in testicular cells. This is also dependent on the HERV Rec protein – a role that may have as yet unknown physiological implications. This uncertainty makes it difficult to interpret high levels of expression of the same viruses and their genes in testicular tumours, though some authors question if dysregulation of cORF expression might contribute to the onset and progression of germ cell tumours.[51] Despite the uncertainty, Nelson and colleagues conclude that the weight of evidence amounts to convincing evidence of the involvement of HERVs and their products in carcinogenesis.[52,53] As we saw with mutation, the difficulty in proving direct causation is due to the involvement of multiple genetic pathways together with an ill-defined interplay between environmental and epigenetic factors in a multistep, complex aetiology.

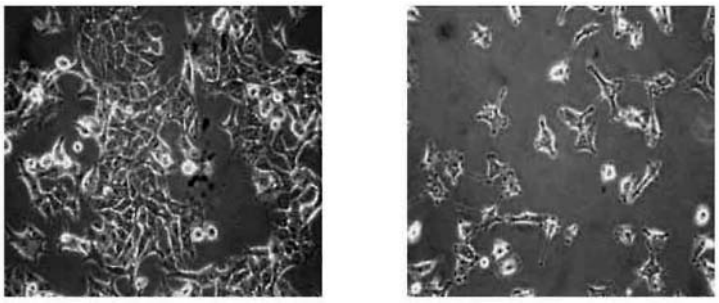
Possible ways in which HERVs might be involved in carcinogenesis are outlined in a helpful review article by Ruprecht and colleagues, with a simplified scheme depicting a theoretical series of steps.[54] An interesting possibility is the potential of HERV proteins to act as tumour recognition antigens, thus provoking an anti-tumour immune response that might be beneficial in immunological surveillance and defence against cancers. Conversely, Manganey and colleagues have confirmed, in a series of studies, that the expression of the *env* protein of both exogenous retroviruses and HERV-H on the surface of tumour cells allows them to evade immune rejection.[55,56] For Ruprecht this adds the potential for 'tumour immune escape' mediated by HERV *env* proteins expressed on tumour cells, which in turn might provoke dysregulation of the normal immunosuppressive role of HERV proteins, such as syncytin-2. In a related study, Ruggieri and colleagues have discovered a novel signal peptide coded by the *env* gene of HERV-K(HML-2), a family of viruses known to be associated with testicular germ cell tumours, raising the possibility that this might also contribute to immune evasion of the tumour cells.[57] Recruitment of the expression of HERV-W syncytin-1 protein in certain breast and endometrial cancers may result in fusions involving cancer-to-endothelial cells, or cancer cell-to-cell fusions, which may promote tumour growth.[54,58]

HERV-related retroposons in carcinogenesis

Where the position of HERVs is believed to be fixed in the chromosomes following endogenization, other HERV-related products, such as LINEs, SINEs and *Alus*, are capable of multiplying themselves and reinserting within the genome. These insertions can result in mutation-like disruption of coding regions, interference with regulation within introns and regulatory sequences, or translocations and deletions through virus-style recombination between homologous and non-homologous chromosomes. Morse and colleagues have reported a tumour-specific rearrangement of a *MYC* oncogene locus brought about by L1 insertion into an intron, which was associated with ductal adenocarcinoma of the breast.[59] Miki and colleagues have reported a disruptive L1 insertion into the *APC* tumour suppressor gene in a case of colon cancer.[60] Given that there are many other tumour suppressor genes, such as *RB* (retinoblastoma), *WT* (Wilm's tumour), *DCC* and *MCC* (colorectal carcinomas), and *p53*, and given that L1 insertions are thought to be widespread and random, these must now be considered as one of the potential mechanisms for carcinogenesis through suppressor gene disruption.

SINEs are of different origins to *Alus*, with which they are often grouped, but both depend on HERVs or LINEs for transposition and they show behavioural similarities. Misra and colleagues have reported the loss of a band of 443 nucleotide bases in tumour DNA taken from a grade IV glioblastoma multiforme, which, on sequencing, was found to comprise a group of SINE-R sequences that were derived from HERV-K.[61] Related sequences were also found in the tumour suppressor gene BRCA2 and the DNA repair gene XRCC1. While this does not demonstrate a cause–effect relationship, it suggests a possible role for the HERV sequences in gene inactivation through viral recombination during carcinogenesis.

Figure 1
Effects of L1 silencing in cancer. The left view shows melanoma cells of the A-375 culture line. The right view shows the same cells after L1 expression was blocked by RNA interference. The clumping of rounded tumour cells has reverted to the more normal stellate forms with dendritic processes. This was accompanied by some normalisation of the genetic processes associated with cell proliferation. Kindly provided by Corrado Spadafora

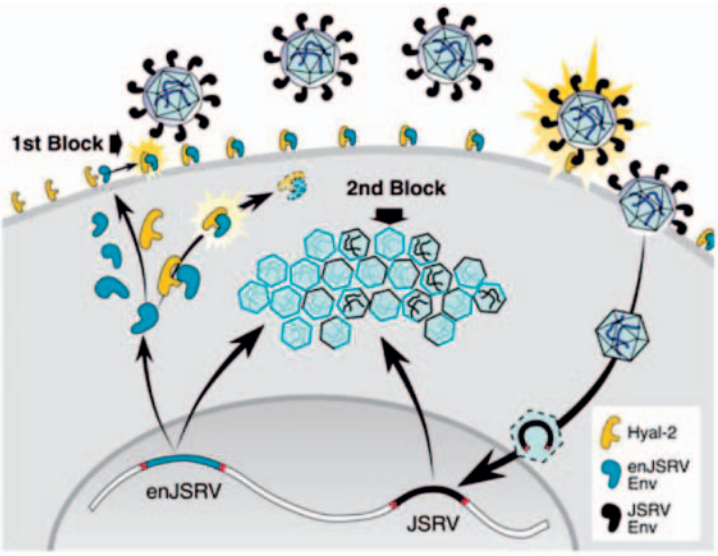


The enormously high frequency of *Alus* in the human genome and the fact that they can interrupt normal genetic function through inserting into coding exons, or into introns, where they cause alternative splicing, or by unequal homologous recombination, means that *Alus* are a major consideration in the genetics of cancer.[62–64] For example O'Neil and her colleagues have shown that the *MYB* oncogene, which is frequently duplicated during the pathogenesis of human T cell acute lymphoblastic leukaemia (T-ALL), is flanked by *Alu* repeats, making it susceptible to tandem duplication as a result of *Alu*-to-*Alu* recombination on homologous chromatids. These duplications occur at low frequency during normal thymocyte development in healthy people, but they are clonally selected during the molecular pathogenesis of human T-ALL, a thymocyte malignancy.[65] Roughly one in four hereditary breast cancers are attributed to mutations affecting *BRCA1* or *BRCA2* genes. Montagna and colleagues describe a 3kb deletion in *BRCA1* in two high-risk families, which encompassed exon 17 and gave rise to a frameshift mutation.[66] The rearrangement was the result of recombination between two very similar *Alu* repeats. This type of mutation would not have been identified by the common methods of detection, based on single exon amplification using PCR. Homologous recombination between *Alu* elements has also been associated with acute myeloid leukaemia, sometimes with a normal karyotype.[67–69] Multiple endocrine neoplasia type 1 is an autosomal dominant cancer syndrome, which is caused by germline mutations of the tumour suppressor gene *MEN1*. Fukuuchi and colleagues have reported an *Alu*-linked germline deletion of the *MEN1* flanked by *Alu* sequences in a family where, again, the deletion would have been undetectable by conventional sequencing analysis.[70] *Alu* repeats have also been associated with familial colorectal cancer,[71,72] breast and ovarian cancer,[73,74] Ewing's sarcoma[75] and glioma,[76] meanwhile it seems likely that this list of associations will increase as the database grows.

Two illustrative approaches to remedial therapy, based on HERVs

Spadafora and colleagues have investigated the effects of blocking reverse transcriptase in a variety of cancers in murine and human cell lines, including teratocarcinomas, fibrosarcoma, osteosarcoma, gliomas, melanoma, and carcinomas of the colon, breast, prostate and thyroid, to show that, regardless of histological origin, RT inhibition caused a dramatic reduction in tumour cell proliferation, meanwhile inducing a more normal cell differentiation, which included a reprogramming of gene expression to a pattern that was more typical of normality.[77,78] They also demonstrated qualitatively different responses *in vitro* and *in vivo* after blocking the effects of the reverse transcriptases of HERV-K and LINE-1 origin, suggesting important differences in function between the two retroviral components. Unlike conventional therapy, these studies showed a novel pattern of reversal of malignant transformation, behaviour and genetic expression in the affected cell lines as well as a marked reduction in the proliferation of human cell tumours in laboratory strains of mice (Figure 1).[79,80] The reduction in cell proliferation and reprogramming of differentiation reverted to malignant behaviour when they removed the anti-RT drug from the cellular or animal models. In a recent study of the process of malignant transformation of melanoma cells, the same authors have reported HERV-K activation during two of the key stages of carcinogenesis: in the initial transformation to malignancy; and in the ability of malignant cells to escape immune detection by the body's surveillance.[81] In cell cultures, these changes were accompanied by vigorous HERV-K expression,

Figure 2
The two sites of enJSRV block. The endogenous JSRV blocks genital invasion by the exogenous virus at two sites, the attachment of the *env* gene at the epithelial cell surface and at the stage of exogenous viral replication in the cytoplasm. Kindly provided by Massimo Palmarini



including massive production of virus-like particles. Reduction of the expression of HERV-K, using RNA interference, prevented some of the changes involved in malignant transformation.

Another pioneering approach has been adopted by Palmarini and colleagues studying Jaagsiekte sheep retrovirus (JSRV), which causes a major infectious epidemic in sheep leading to fatal ovine pulmonary adenocarcinoma.[82] This is of specific interest to the study of carcinogenesis since, uniquely among the known retroviruses, the JSRV *env* gene is directly oncogenic, inducing cancerous transformation of the alveolar type II cells and non-ciliated bronchiolar cells (Clara cells) in the lungs of infected sheep as well as inducing cancer in a number of cell lines *in vitro*.[83] The exogenous JSRV is closely related to the endogenous form of the same virus, enJSRV, which is expressed at high level in the genital tract of the ewe. The original portal of entry was surely genital, but Palmarini's group have shown that expression of a specific genetic locus derived from the *gag* protein of the endogenous virus (the enJS56A1 locus) now blocks viral entry and replication in the cells lining the genital tract, thus preventing genital transmission.[84] Meanwhile the exogenous virus has evolved a new transmission strategy, via pulmonary infection. Palmarini and his colleagues have been studying the molecular basis of the enJS56A1 block, with the aim of inducing expression of the enJS56A1 locus within the cells lining the pulmonary tract in germ line experiments (Figure 2). In essence they are looking at the possibility of inducing ERV-induced genetic change in sheep that would promote species resistance to the exogenous virus, a novel approach to a carcinogenic retroviral epidemic in a mammal.

In summary

Although our current understanding of the contribution of HERVs to carcinogenesis is limited by our lack of understanding of their normal physiological roles, it would appear that they play significant, and very likely, a variety of different roles in both anti-cancer protection and pathogenesis. It is important that future mass genetic screening systems should include the possibility of HERVs and related genetic sequences in their programmes. A fuller understanding of this HERV role, coupled with a deeper epigenetic understanding, is likely to contribute to new lines of therapy, including immunotherapy.[25,85]

Part 5 of this series will examine the role of epigenetics and genomic duplications in health and disease.

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