Endogenous Retroviruses and the Development of Cancer

George Kassiotis

J Immunol 2014; 192:1343-1349; doi: 10.4049/jimmunol.1302972
http://www.jimmunol.org/content/192/4/1343

References
This article cites 87 articles, 31 of which you can access for free at:
http://www.jimmunol.org/content/192/4/1343.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Endogenous Retroviruses and the Development of Cancer

George Kassiotis

Mammalian genomes include a considerable number of endogenous retroviruses (ERVs), relics of ancestral infectious retroviruses, whose proviruses have invaded the germ-line. The documented ability of infectious retroviruses to cause cancer has greatly contributed to the discovery of ERVs. It also reinforced the concept that ERVs are causative agents of many cancers, a notion that historically has not always stood up to experimental scrutiny. The recent greater appreciation of the complexity of ERV biology and the identification of dedicated host mechanisms controlling ERV activity have revealed novel interactions between ERVs and their hosts, with the potential to cause or contribute to disease. In this review, the involvement of ERVs in cancer initiation and progression is discussed, as well as their contribution to our understanding of the process of transformation and to the invention of innovative preventive and therapeutic cancer treatments. The Journal of Immunology, 2014, 192: 1343–1349.

Ultimately, germ-line-integrated ERV sequences degenerate by accumulation of mutations or recombination events, which destroy their ability to produce infectious virus (1, 10). For example, recombination between the two LTRs excises the whole provirus, leaving only a single LTR (referred to as solo LTR, Fig. 1). These make up the majority (90%) of all LTR elements. ERVs that have recently invaded the germ-line are more likely to represent full, sometimes infectious, copies and are also more likely to be insertionally polymorphic between individuals. Inversely, older ERVs have sustained significantly more damage to their open reading frames (ORFs), rendering them replication defective, and they also have reached genetic fixation in their host genome (10).

It is important to note that the ability of ERVs to produce infectious virions differs substantially between host species. For example, replication-competent endogenous copies of murine leukemia virus (MLV) or mouse mammary tumor virus (MMTV) can be found in many strains of laboratory mouse (4). Infectious viruses produced by these ERVs can cause new somatic cell infections, as well as transmit vertically to progeny as fully exogenous retroviruses. Replication-competent ERVs have also been found in other species of mammal (11). However, no replication-competent human ERV (HERV) has been found. HERV ORFs appear to be more defective than murine ERVs, and most HERVs are fixed in the human germ-line. One possible exception is the HML2 group of the HERV-K family, which is considered the most recently acquired family (12–14). HERV-K(HML2) proviruses have the most intact ORFs, although none seems to be replication competent, and at least some of them display insertional polymorphism within the human population (12–14). In addition to ERVs, non-LTR retrotransposons are still active in both humans and mice, where frequent somatic retrotransposition can be demonstrated (8, 13, 15).

Given the ability of ERVs and other retroelements to move in the genome, it is perhaps anticipated that these “genomic parasites” have been incriminated in the pathological processes leading to cancer, which is also viewed as a “genomic disease.” Understanding the nature of ERV association with cancer and elucidating the precise mechanisms underlying any such association will be important in reaching a verdict.
ERVs and cancer: a historical perspective

Historically, ERVs have been linked to the development of cancer as a result of the transforming nature of retroviral infection, a property that was also a major contributor to the discovery of ERVs (11, 16, 17). Furthermore, the study of retroviral infection in animal models has greatly contributed to the delineation of the process of cellular transformation and laid the foundation of modern cancer research (18).

The one animal that supported the study of retroviral infection and discovery of ERVs, as well as their association with cancer, is arguably the mouse (11, 16, 17). Records of spontaneous tumors observed in mice date back to the 1890s and include mice that were caught in the wild or supplied by mouse fanciers, who had been breeding selected phenotypic traits (16). Cancer research scientists both in Europe and North America around that time had been obtaining mice from mouse farms, which often kept family records of the selected breeds (16). This collaboration fertilized the early notion of “heritable” cancer, which was later investigated extensively at the Bussey Institute of Harvard University in Boston, Massachusetts. There, C.C. Little and his mentor, W.E. Castle, carried out seminal work on cancer genetics with mice likely originally obtained from a nearby mouse farm run by Miss A. Lathrop, who had also made important observations on the spontaneous incidence of cancers in her mice (19, 20). This work at the Bussey Institute also included the genetics of coat color and transplant rejection and generated the first inbred mouse strains, which proved an invaluable tool not only in the dissection of cancer genetics and transplantation, but also later in all other fields of experimental biology (20).

Little’s work also led to the discovery by J.J. Bittner at the Jackson Laboratory in Bar Harbor, Maine of the “milk factor,” a vertically transmitted extrachromosomal agent causing mammary cancers in mice (16). This, of course, was MMTV, the prototypic β-retrovirus responsible for the majority of mammary tumors in mice, and its transmission via milk seemed to argue against Mendelian cancer inheritance. However, several years later, the Dutch investigators P. Bentvelzen and J.H. Daams discovered germ-line–integrated copies of MMTV proviruses, which were indeed inherited as Mendelian traits in the GR mouse strain and that encoded fully infectious MMTV (16, 17).

Also notable are the independent parallel discoveries of endogenous copies of MLV in inbred mouse strains and of avian leukosis virus in domestic fowl. The latter work by R. Weiss in London, England was one of the earliest suggestions of the existence of ERVs and is reviewed elsewhere (11, 17). The leukemogenic potential of these ERVs also reinforced the association with cancer. However, at least for the laboratory mouse, ERVs appear to have far-reaching effects. First, many murine ERVs are still very much active and, thus, a significant source of mutation of the murine germ-line. It is estimated that 1 in 10 spontaneous phenotypes that have been described in mice are caused by insertional mutagenesis by an ERV (21, 22). Second, active ERV retrotransposition in the mouse germ-line and intensive inbreeding involved in the establishment of laboratory mice have resulted in extensive insertional ERV polymorphisms among mouse strains (4). Insertional ERV polymorphism is a considerable part of all structural variants among mouse strains identified by genome sequence comparisons and undoubtedly will affect the phenotypic outcome of a given single-gene mutation in distinct inbred mouse strains (23).

Similarly to the first inbred mouse strain, DBA, bred for and named after its coat color, which is the result of a germ-line integration of an MLV provirus (24), selection of certain phenotypic characteristics by early mouse fanciers and experimental biologists may have inadvertently contributed to the high ERV activity in laboratory mice, rendered polymorphic by subsequent inbreeding. Although this situation is ideal for the study of ERVs in particular, it is something that should be considered in the interpretation of studies involving mice.

Potential mechanistic links between ERVs and cancer

With the possible exception of rare acutely oncogenic variants, exogenous infectious retroviruses cause cancer principally by insertional mutagenesis, a process that has led to the identification of numerous cellular oncogenes or tumor-suppressor genes (16, 18). This well-established transforming potential of exogenous retroviruses is also shared by at least those ERVs that retain infectivity, such as the infectious copies of MMTV or MLV in certain mouse strains (3, 4, 16, 18). However, the only contribution of the germ-line copy of these infectious retroviruses is transmission to progeny, which, in the case of both MMTV and MLV, can also be achieved by vertical transmission via milk. Thus, the germ-line copies of infectious ERVs may not directly participate in the process of cellular transformation.
transformation. Instead, it is new somatic integrations as a result of cellular infection with virions encoded by infectious ERVs that mediate the mutagenic effect. However, in contrast to mechanisms necessitating somatic infections by infectious retroviruses, the sheer number and repetitive nature of ERVs in the genome and, consequently, their presence in every cell may trigger additional pathogenic mechanisms that do not require the production of infectious virions (25, 26); these are discussed separately (Fig. 2).

Transformation by insertional mutagenesis. The process of transformation by endogenous copies of infectious MMTV and MLV almost invariably involves insertional activation of oncogenes, such as Wnt1 or Notch1, among many others, or disruption of tumor-suppressor genes, and it has been described extensively in the mouse (27). However, the search for infectious retroviruses, endogenous or otherwise, which may be causing cancer in humans through a similar mechanism, was not free from controversy (28). The strong link between MMTV infection and mammary tumor in mice inspired an exhaustive, but as yet unsuccessful, quest for a retrovirus causing human breast cancer. The lack of convincing evidence, despite major efforts in identifying human tumor viruses, led to scientists referring to the elusive target as “rumor viruses” (28).

Although the discovery of human T-lymphotropic virus 1 unequivocally proved the existence of a tumor-inducing exogenous human retrovirus, no HERV proviruses with the potential to produce an infectious retrovirus has been discovered. One of the most convincing evidence for infectious HERVs would be finding new somatic HERV integrations, and genome sequencing and analysis tools are sufficiently developed today to provide a certain degree of validity, even of negative findings. In this regard, data from cancer genome sequencing identified >180 LINE-1 somatic integrations in human cancer cells (29). In contrast, the same data identified only a single somatic integration of a small HERV fragment, which was likely the result of a microhomology-mediated DNA repair mechanism rather than genuine retrotransposition (29).

Nevertheless, it is conceptually possible that infectious retroviruses derived from HERV precursors do arise spontaneously. These may not cause generalized infection within an individual or even transmission between individuals and, therefore, would elude detection. Indeed, the HERV-K (HML2) family comprises insertional polymorphic proviruses with various degrees of inactivation that collectively, but not individually, contain the complete information for a replication-competent recombinant retrovirus (30, 31). Recombination between defective HERV-K(HML2) proviruses was shown to occur naturally (14, 32) and may be facilitated by exogenous retroviruses, as in the case of HIV-1 infection (33).

Although such recombination events restoring ERV infectivity would be exceptionally rare, recent mouse studies (34, 35) showed that the probability of infectious MLVs arising from defective ERV precursors can be significantly elevated when innate and adaptive immune responses are defective. This process ultimately leads to development of leukemias in mice and additionally seems to be controlled by environmental parameters, such as the intestinal microbiota (34). Therefore, it is possible that infectious HERVs do arise and contribute to cancer development in specific situations involving immunodeficiency.

Tumor-promoting retroviral proteins. Although most, if not all, ERVs have accumulated replication-inactivating mutations, many still contain intact ORFs that produce retroviral proteins (36, 37). These ERV-derived proteins may serve as a source of Ag for the immune system and, for certain ERVs, also a source of superantigen, with examples including MMTV in mice (38) and HERV-K18 in humans (39). ERV-encoded proteins are unlikely to be distinguished by the immune system from all other host proteins and, thus, are expected to contribute to T or B cell AgR repertoire-selection processes, as well as peripheral regulatory mechanisms, as part of “self” (40, 41). Nevertheless, immune reactivity to ERV products can often occur spontaneously in infection or cancer and is considered the driving force of several autoimmune disorders in mice (42). Immune reactivity to ERV products can also be induced experimentally in mice and nonhuman primates (43, 44), suggesting that immunological

![FIGURE 2. Potential mechanisms of ERV-mediated transformation.](http://www.jimmunol.org/)

Chromosomal aberrations

- Host DNA
- provirus
- Chromosomal rearrangements

Insertional mutagenesis

- Host gene
- provirus
- Insertional mutagenesis

LTR function

- Host gene
- Repressed state
- Gene transcription

Replication intermediates

- Host gene
- Repressed state
- Gene transcription
- mRNA regulation

Retroviral protein expression

- Host gene
- Repressed state
- Gene transcription
- mRNA regulation
- Immunosuppression
- Cell fusion/metastasis

Downloaded from http://www.jimmunol.org/ by guest on April 15, 2017
tolerance to ERV-derived proteins is not complete. ERV proteins may also retain biological function, which was proposed to contribute to cellular transformation (45). Indeed, expression of HERV-encoded proteins has been detected, by Ab staining, in a variety of human cancers (25). Although certain HERV proteins can also be expressed in healthy, nontransformed tissue, some show exclusive expression in tumors (25, 28). However, direct mechanisms of contribution to cancer development have been proposed for only few of these proteins.

Examples include two accessory proteins, Rec and Np9, produced as alternative splicing products of the HERV-K (HML2) env gene. These proteins are frequently found in transformed cells from testicular germ cell tumors but not in healthy cells (25). Rec, in particular, interacts with the cellular transcription factor PLZF, and its overexpression in mice can induce early seminomas (46).

The envelope proteins of several ERVs may also contribute to the development of cancer through their fusogenic or immunosuppressive properties. Both properties of expressed retroviral envelope proteins can also serve physiological processes in the host (3). For example, products of the host syncytin genes, which are derived from retroviral env genes, are essential for placenta, by mediating cell fusion of syncytial cell layers, and for maternal tolerance of the fetus, by immunosuppression (47). Expression of syncytins, which is normally restricted to the placenta, was found in human cancer, and it is hypothesized that syncytin-mediated cell fusion participates in transformation or metastasis (48, 49).

Seminal work by T. Heidmann further defined the immunosuppressive domain within the transmembrane subunit of the envelope protein of several retroviruses, as well as its potential role in tumor growth (50). Overexpression of Moloney MLV transmembrane subunit in murine tumor cell lines led to tumor growth in recipient mice that would otherwise immunologically reject them (51). Equally, knockdown of env transcripts in B16 melanoma and Neuro-2a neuroblastoma cell lines, both of which produce infectious MLVs derived from ERV precursors, rendered them susceptible to immune rejection in vivo (52, 53). Thus, ERV-encoded immunosuppressive domains may contribute to cancer development by establishing the immunosuppression necessary for tumor growth and, in addition to syncytins, the env of HERV-E (54) and HERV-H (55) was shown to possess immunosuppressive potential.

**ERV effects on genome function.** For every ERV with the ability to produce proteins or virions, there are many more that are transcriptionally silenced or may produce only noncoding RNA transcripts. Both noncoding RNA transcription from ERVs and their repetitive presence in the genome as DNA elements have enormous potential to affect genome function (56). Although some of the complex effects of ERVs on genome function benefit the host and have been co-opted during evolution, others may be pathogenic (56, 57).

The cell possesses an array of enzymes involved in DNA metabolism, and some of these are considered essential for preventing accumulation of nucleic acid intermediates from incomplete ERV replication cycles. These include TREX1, an ssDNA exonuclease, and SAMHD1, an enzyme involved in the regulation of cytosolic deoxyribo-NTPs (58) that may also exert nuclease activity (59, 60). These enzymes interfere with the replication of exogenous retroviruses and may have similar roles against ERVs (59, 60). This potential role against ERVs is thought to be at the center of the pathogenic mechanism initiated when these pathways are not operative. Indeed, mutations in the genes encoding these enzymes lead to autoimmune or autoinflammatory human conditions associated with elevated production of type I IFNs (61). Notably, SAMHD1 mutations were also identified in a variety of human cancers (62–64), suggesting that SAMHD1 is a tumor-suppressor gene, although the precise mechanism connecting SAMHD1 mutations with cancer development is not clear.

In addition to the effect of generic ERV transcripts and replication intermediates, noncoding RNAs of certain ERVs can have more direct effects on genome function. Repression of several proto- oncogenes, as well as other cellular genes, relies on the premRNA splicing factor PSF (65). In turn, PSF activity is negatively regulated by binding to noncoding RNAs, one of which is transcribed from a HERV-K11 provirus (66), thus potentially linking HERV transcription with protooncogene activation.

ERVs additionally influence genome function without producing an RNA transcript (57, 67–69). They provide promoter activity, numerous binding sites for transcription or other DNA binding factors, or homology sites for DNA recombination. Indeed, ERV LTRs are perfect docking sites for DNA-binding factors, which are critically involved in cancer development (2). One such factor is p53, an important tumor suppressor maintaining genome integrity and preventing mutation, often referred to as the “guardian of the genome” (70). One in three of all p53 binding sites identified in a human cancer cell line were within the LTRs of two HERV families, which, when bound by p53, could activate transcription of downstream genes (71). Thus, ERV LTRs may also participate in the tumor-suppressing p53 response.

However, ERV LTRs may also promote cellular transformation by in cis activation of downstream oncogenes. Transcriptional derepression of the CSF1R gene, encoding CSF-1 receptor, by a demethylated MalR LTR acting as an alternative promoter was linked with survival of cancer B cells in Hodgkin’s lymphoma (72). This study revealed how loss of epigenetic silencing of retroelement LTRs contributes directly to tumor growth.

Lastly, the repetitive nature of ERVs provides an ideal substrate for nonallelic homologous DNA recombination (2, 32, 56), which results in a variety of chromosomal rearrangements. Recombination between ERVs is responsible for notable germ-line genomic rearrangements leading to known human genetic disorders (32). Such recombination events can also occur in somatic cells, resulting in chromosomal translocations, which, in turn, underpin cellular transformation. Examples include recurrent chromosomal translocations in human prostate cancer, which create fusions between a HERV-K provirus and an otherwise dormant oncogene of the ETS (E26 transformation–specific) family (73). Further study of chromosomal translocations in cancer is expected to add to the list of ERVs driving these translocations.

**Elevated ERV activity in cancers: harmful cause or useful consequence?**

It is evident that ERVs possess properties that might cause or contribute to cancer development, and the activity of many ERVs is frequently elevated in transformed cells. There are
numerous examples of ERV protein expression or virion production in mouse and human cancer cells (74, 75). However, both ERV proteins and virions also can be seen in either certain healthy tissues, such as the placenta (76–78), or in infection and other nonneoplastic diseases. Therefore, elevated ERV activity, even if restricted to cancer cells, should not be taken to signify causality. Rather, most of this activity is likely to represent lack of ERV regulation under these conditions.

ERV activity in a cell is regulated by both cell-intrinsic factors and external signals (1, 3, 34, 79). Epigenetic silencing seems to be a major way in which the cell prevents transcription of repetitive elements, including ERVs (1, 3). Global DNA hypomethylation, leading to epigenetic derepression, is a hallmark of cellular transformation and a major contributor to oncogene activation. In the altered epigenetic landscape of transformed cells, ERV derepression should be expected as a consequence (80).

Nevertheless, causal or otherwise, characteristic patterns of ERV expression are often seen in various tumors (25), offering a unique approach to immunotherapy, trials of which already have been contemplated (44). Largely tumor-restricted expression was proposed for several ERVs, including HERV-K (HML6) in melanomas, HERV-K(HML2) in germ-cell carcinomas, HERV-E in renal cell carcinomas, or even syncytin-1 in diverse cancers (81, 82). HERV expression in some of these cases also was demonstrated to lead to immune reactivity against HERV-derived epitopes (83, 84). Of note, hematopoietic stem cell transplantation in patients with renal cell carcinoma resulted in tumor regression, likely due to a graft-versus-tumor effect. In such patients, antitumor CTLs were found to be targeting a HERV-E–encoded epitope (84), demonstrating the potential for anti-ERV immune responses in tumor regression.

Tumor immunotherapy by anti-ERV CTL responses also can be experimentally demonstrated in mice. CTL epitopes were described in EL-4 lymphoma cells and C26 colon carcinoma encoded by an endogenous MMTV and MLV, respectively (85, 86). Immune priming with a peptide from the envelope protein of Emv2, an endogenous defective MLV in C57BL/6 mice, leads to eradication of syngeneic B16 melanoma cells, which also express the same MLV envelope (43). B16 cells are also known to produce recombinant MLVs, which have restored Emv2 infectivity and have reinfected these cells (87). Infectious MLVs produced by tumor cells and spreading in the host will inevitably complicate the interpretation of immunological studies if tumor transplantation is accompanied by retroviral infection of the host. It would appear that many, if not most, of the transplantable tumor cells carry infectious retroviruses. Some are the result of recombination between defective endogenous precursors, as in B16 and Neuro-2a cells, which are likely linked to the original transformation event (53, 87, 88). Others may be unwittingly the combination between defective endogenous precursors, as in cells carry infectious retroviruses. Some are the result of recombination between defective endogenous precursors, as in B16 and Neuro-2a cells, which are likely linked to the original transformation event (53, 87, 88). Others may be unwittingly the combination between defective endogenous precursors, as in

Conclusions

The tumorigenic mechanisms used by exogenous retroviruses, namely insertional mutagenesis and acquisition of oncogenes, helped to pave the way to understanding cellular-transformation events. This increased understanding of the complex processes of transformation no longer required infection with retroviruses, because similar genomic insults are generated by a variety of other mechanism. However, this increased understanding of genome structure and function has also highlighted the potential importance of ERVs, the ultimate genomic parasites, although obtaining compelling evidence remains a challenge. Just as their predecessors, exogenous retroviruses, did in the past, ERVs may illuminate additional mechanistic details of cancer initiation and progression, as well as offer new prospects for cancer immunotherapy.

Disclosures

The author has no financial conflicts of interest.

References


