Vol 2:9-14

JEMI-PEARLS

Human Endogenous Retroviruses: Role in Human Genome Evolution, Implications in Disease, and Potential for Therapeutics

Alan Stachowiak
Department of Microbiology and Immunology, University of British Columbia

BACKGROUND INFORMATION

Endogenous Retroviruses (ERVs) are elements in the genomes of all currently studied eukaryotic organisms that can be derived from retroviruses [1]. In humans, these elements comprise 5 to 8% of the genome and are called Human Endogenous Retroviruses (HERVs) [1]. Retroviruses regularly infect somatic cells and integrate their genome into the host's DNA as part of their replication cycle. Rarely, a retrovirus can infect a germline cell, which will then go on to develop into a mature organism. This organism will carry the retroviral sequence as part of its genome and can pass it on to its offspring as a new allele. Researchers have also proposed that the retroviral sequences evolved from transposons, specifically retrotransposons, meaning that these sequences became exogenous and may have been the source of the retroviruses they resemble [2]. Most endogenous retrovirus sequences have nonsense mutations or major deletions resulting in defective protein products or no protein products being produced [3]. These mutations and excisions occur due to evolutionary pressure to ensure that nearby human genes remain unaffected [3]. However, a few families of HERVs are active, producing functional protein products and even full virions [4].

We share most of our endogenous retroviruses with chimpanzees, apes and old world monkeys such as macaques, as determined through sequence alignments with chimpanzees [5]. Less than 100 human-specific ERVs (0.1% of the human genome) were found as a result of that study [5]. This suggests that the majority of the ERVs were inserted into our genome before the last common ancestor of humans and chimpanzees, which lived around 5 to 10 million years ago [6]. In addition, we cannot determine the age of ancient ERV insertions since random point mutations accumulate over time, leading to our inability to find homologues in other species beyond about 60–80 million years of age [1]. However, all currently studied eukaryotic organisms, including useful models such as mice,

Received: Dec./15 **Accepted:** Dec./15 **Published:** July/15

Citation Stachowiak, A. 2017. Human Endogenous Retroviruses: Role in Human Genome Evolution, Implications in Disease, and Potential for Therapeutics. JEMI-PEARLS. 2:9-14.

Caenorhabditis elegans, and *Drosophila* flies bear ERVs in their genomes [1].

HERVs are retroviruses and have varying degrees of similarity to exogenous retroviruses. Class I HERVs such as HERV-W and HERV-FRD resemble gammaretroviruses, whereas class III HERV-L more closely resembles lentiviruses [4]. The newest integration into the human genome (within the last few hundred thousand years), the HERV-K family, resembles betaretroviruses and serves as a model for HERV genomic organization [4] (Fig. 1). The genome encodes a polyprotein containing the *gag* region (core and structural proteins), *pro* region (protease), *pol* region (reverse transcriptase and integrase), *env* region (coat proteins) and *rec/np9* region. The Rec/np9 proteins are involved in the release of late-phase viral mRNAs from the nucleus of the host cell, as well as the downregulation of host nuclear mRNA release [4]. The sequence encoding the polyprotein is flanked by long terminal repeat (LTR) segments.

> “Research into the different facets of HERV activity in our bodies could lead to the development of novel therapeutics to combat complex diseases associated with endogenous viruses”

RESEARCH QUESTIONS

As HERVs have been a part of the human genome for millions of years, they have gained functions through evolution, and more recently have been implicated in disease [4, 11]. This new field of research could provide us with insight into genome evolution, as well as the causes of multifactorial diseases associated with endogenous retroviruses. Research into the different facets of HERV activity in our bodies could lead to the development of novel therapeutics to combat complex diseases associated with endogenous viruses, many of which do not currently have effective cures.

There are three critical overarching research questions that need to be explored in the field of HERVs for significant progress to be made in the fight against HERV-associated diseases. Firstly, their roles in human genome evolution, specifically the functions of HERV protein products during human development as well as LTR promoter and enhancer effects, need to be elucidated. Secondly, the role of HERVs in disease needs to be established, using its putative role in ALS pathogenesis as a model for research into the harmful effects of HERVs. Finally, utilizing this information, this review aims to explore the potential for novel therapeutics against HERV-associated diseases.

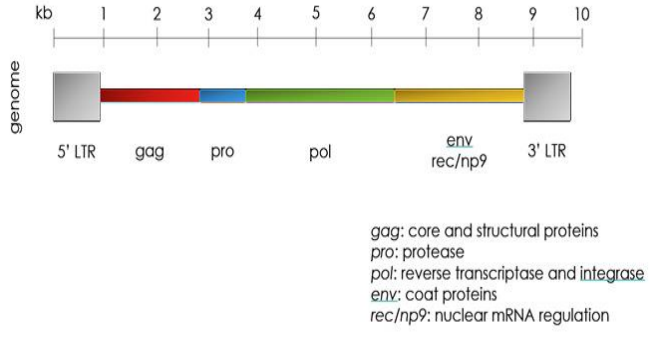

FIG. 1 Genome Organization of HERV-K.

PROJECT NARRATIVE

What is the role of HERVs in human genome evolution?

The majority of the ERVs in our genome have been present since our evolution from the last common ancestor of humans and chimpanzees [6]. The sequences encoding these ERVs have been involved in shaping our genome by acting as promoters and enhancers for nearby human genes [7]. Specifically, when the LTR regions are inserted into the 5′ UTR of protein coding genes, they can promote the transcription of these human genes [7] up to 70–100 kb away [8]. The vast majority of these LTRs act in the sense direction to their corresponding genes, but some have been shown to act in an antisense direction or even as a bidirectional promoter [9]. LTRs acting as promoters for human genes influence gene transcription rates but, importantly, have also been shown to influence tissue specificity [10]. For example, the tissue-specific promoter of salivary amylase is composed of an HERV LTR [10]. Excision of the HERV from the promoter results in reversion to expression solely in the pancreas [10].

HERVs are active in the reproductive system more than anywhere else in the body. This is due to two factors. Firstly, many reproductive gene promoters are LTRs which is thought to be due to a lack of DNA methylation in germline tissues [11]. Secondly, a variety of HERV proteins as well as full virions can be expressed in these immune-privileged areas of the body [12]. Specifically, HERV-W, HERV-FRD and ERV-3 envelope proteins are highly expressed in the placenta [12]. These proteins are important to placental development and are now thought to be necessary for proper reproductive function [12]. Evidence has shown that these proteins, called syncitin, facilitate the fusion of cytotrophoblast cells to form a multinucleated layer called the syncytium of the placenta [12]. The syncytial layer is a microscopically thin and confluent tissue layer which forms the physiological barrier between maternal and fetal circulations (Fig. 2). All O_2 and nutrients from the mother as well as CO_2 and waste from the fetus must be exchanged through this syncytial layer (Fig. 2). The syncytium also serves to prevent maternal immune rejection of the fetus, which inherits half of its antigens from the father as the multinucleated layer does not allow maternal white blood cells to travel to the fetus [12] . This immune barrier suggests that HERVs may have been positively selected to contribute to the evolution of viviparity (mammals birthing without the use of eggs) [12].

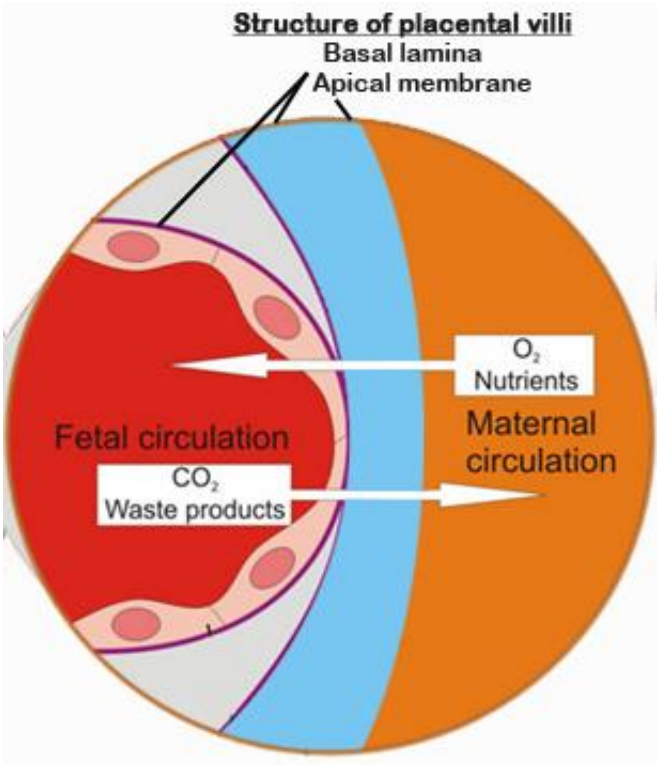

FIG. 2 Syncitiotrophoblast and Uteral Environment. Adapted from the Professional Institute of Medical Excellence Database.

Finally, HERVs are very prone to recombination events with other inserted viral elements, leading to genome plasticity [13]. There are sections of the genome with a higher than average number of inserted HERV elements, such as within the major histocompatibility complex (MHC) immunohaplotype blocks [13]. Studies have shown that these MHC HERV elements can recombine, leading researchers to hypothesize that these HERV elements have facilitated the recognition of many more antigen types than would have been possible with a genome deprived of HERVs [13]. However, recombination events between HERV elements can lead to microdeletions which are associated with genetic diseases [14]. Through LTR promoters, expressed proteins and recombination, HERVs have significantly influenced human genome evolution and development. However, these additions have also established the potential for disease.

What is the role of HERVs in disease?

HERV protein product overexpression has been implicated in a variety of diseases such as autoimmune syndromes including Multiple Sclerosis (MS) and Rheumatoid Arthritis (RA), as well as in certain cancers [4]. The link between autoimmune diseases and HERVs has been characterized by the overexpression of envelope proteins and their effects on cells implicated in the disease [4]. On the other hand, Gag, Env, Np9 and RT HERV proteins have been implicated as oncoproteins in certain cancers [4]. To illustrate the putative role of HERVs in disease, Amyotrophic Lateral Sclerosis (ALS) will be explored as an example disease in which HERVs seem to play an important role in pathogenesis.

ALS is a progressive, ultimately fatal neurodegenerative disease. It is characterized by the death of neurons resulting in a loss of motor function and concluding with the inability to utilize the muscles necessary for breathing [15]. Following an unsuccessful attempt to link ALS with exogenous retroviruses, research was turned towards HERVs [16]. Li *et al.* investigated the relationship between HERVs and ALS and found many correlations [17].

HERV-K Gag, Pol and Env proteins were expressed in the brains and spinal cords of recently deceased stochastic cases of ALS (no genetic ALS cases were studied) [17]. *In vitro* expression of HERV-K Env in human neuronal cultures resulted in neurotoxicity [17]. *In vivo* expression (using a transgenic mouse expressing HERV-K Env in its neurons) caused degeneration of

motor neurons and subsequent loss of motor function in an ALS-like manner [17]. In the transgenic animals tested, evidence of HERV-K damage included double stranded DNA breaks in neurons as well as nucleolar neuron dysfunction, both of which have been observed in ALS patients [17]. After demonstrating the elevated presence of HERV-K in ALS pathogenesis, the key finding in the study was that HERV-K expression is unlikely to be a response to neurodegeneration for two reasons: the brains of Alzheimer's, Parkinson's, and accidental brain damage patients did not have elevated HERV-K expression, and the treatment of neurons in culture with ALS-inducing toxins did not induce the expression of HERV-K [17]. Many studies associating HERVs with disease present correlational data alone; these findings, showing that HERV expression is unlikely to be a result of the disease itself, are an important step towards proving causation of disease pathogenesis.

Further research is needed on the role of HERVs in disease, using larger sample sizes as well as providing mechanistic evidence to rule out the possibility that the HERVs are overexpressed due to the disease itself, allowing us move away from correlation towards causation.

What is the role of HERVs in potential therapeutics?

Since HERVs have been implicated in numerous diseases, research has recently explored their use as a novel target for potential therapeutics. Two prominent avenues are currently being explored: the treatment of autoimmune diseases using antiretroviral medications, and the use of overexpressed HERV protein products as novel vaccine targets for cancer and HIV treatment [18–20].

Antiviral medications are being explored as HERVs have repeatedly been shown to be overexpressed in a variety of diseases. The presence of HERV elements in disease not only suggests a possible role in pathogenesis but also suggests that researchers may be able to use them as diagnostic biomarkers. Specifically, MS has been associated with high HERV-K Env glycoprotein expression, and one patient who was also diagnosed with HIV unexpectedly recovered from MS after undergoing treatment for HIV [18]. This one patient's case report in 2011 reported that after the patient was started on Antiretroviral Therapy (ART), his MS symptoms disappeared completely and remained absent for more than 12 years of follow up [18]. It was hypothesized that the ART drugs used for HIV may have been coincidentally treating or preventing the progression of MS through the suppression of endogenous HERV-K Env proteins [18]. A record-linked database study of English patients was conducted to determine if patients being treated for HIV were universally less likely to develop MS [21]. The study found that HIV infection is associated with a significantly decreased risk of developing MS [21]. However, mechanisms of this observed and possibly protective association may include the antiretroviral medications incidentally acting to downregulate HERV presence as well as the immunosuppression induced by chronic HIV infection. More studies need to be conducted to elucidate the true nature of the protective effect of antiretrovirals against HERVs and MS.

HERVs can be used as biomarkers in disease and may therefore be used as possible vaccine targets. HERVs seem to be preferentially expressed in diseased, cancerous, and HIV infected cells [19, 20]. Therefore, not only are they promising targets for detection, but researchers have proposed that they may be used to elicit a cytotoxic T-cell (CTL) response through a vaccine [19, 20]. A vaccine against HERV antigens that are typically not expressed in healthy tissues could elicit long lasting CTL attacks specifically against affected cells. Since HERVs are rarely prone to mutation in the timescales relevant to clinical treatment, they could provide stable targets for our body's defenses, compared to rapidly mutating HIV particles or cancer cells that may not have many other unique markers [19, 20]. However, before the creation of a vaccine against HERVs, several issues need to be addressed. Firstly, the specific HERV proteins expressed by cancer and HIV infected cells need to be determined. Secondly, the amount of bystander effect needs to be elucidated, meaning the amount of damage a vaccine would do to healthy cells also expressing HERVs. Finally, researchers need to determine if the pattern of HERVs is similar enough on all the types of cells that HIV infects, or in all cancers [20]. If these research questions can be resolved, a vaccine against HERVs to impede the pathogenesis of a disease may become a possibility.

SUMMARY AND CONCLUSION

HERVs are an integral part of the human genome. They have clearly been shown to be important in terms of tissue specificity and as promoters and enhancers for human genes. Their role in human reproduction and MHC immunohaplotype evolution is also vital to human survival. Despite acknowledging its beneficial role, the idea that HERVs cause disease in humans is still considered controversial by researchers in the field. Much of the basic research on the link between HERVs and disease is correlational, with the possibility of a

confounding factor leading to increased HERV protein expression. Perhaps generalized responses to disease such as inflammation or changed cytokine expression coincidentally lead to greater HERV expression, either near the disease location or throughout the body. However, alternative explanations to the idea that HERVs are involved in human disease are unsurprising considering the fact that medical scientists have shown resistance to the idea of a viral cause for chronic autoimmune diseases.

The direct role of HERVs in causing pathogenesis is, however, seeing mounting evidence in its favour. This role in ALS has been elucidated to a degree, with the important finding being that HERV expression is unlikely to be a response to neurodegeneration. This is pushing research away from correlation and is beginning to show the direct toxicity that HERV protein products can cause. Given such concrete links, more basic research needs to be conducted to elucidate the role of HERVs in many diseases. If pathogenesis experts begin to provide evidence for these links, treatments such as the promising ART treatment of MS patients can begin to receive needed attention.

Regardless of the direct role of HERVs in causing pathogenesis, using them as biomarkers and as targets for vaccines is another promising area of research. Targeting endogenous instead of exogenous elements could provide us with a radical new way to deal with disease. As many HERV-linked diseases (e.g. MS, RA) do not have adequate treatments and/or cures, a vaccine promoting a targeted CTL response against them could revolutionize our ability to treat these illnesses.

ACKNOWLEDGEMENTS

I would like to thank Dr. Jean and my peers for their immense help and feedback throughout the development of this project.

REFERENCES

1. Tristem M. 2000. Identification and characterization of novel human endogenous retrovirus families by phylogenetic screening of the human genome mapping project database. J Virol 74:3715–30.
2. Cotton J. 2001. Retroviruses from retrotransposons. Genome Biology 2:reports0006.
3. Belshaw R, Pereira V, Katzourakis A, Talbot G, Paces J, Burt A, Tristem M. 2004. Long-term reinfection of the human genome by endogenous retroviruses. Proc Natl Acad Sci USA 101:4894–9.
4. Hohn O, Hanke K, Bannert N. 2013. HERV-K (HML-2), the best preserved family of HERVs: endogenization, expression, and implications in health and disease. Front Oncol. 3: 246.
5. Mikkelsen TS, Hillier LW, Eichler EE, Zody MC, Jaffe DB, Yang S, Enard W, Hellmann I, Lindblad-Toh K, Altheide TK et al. 2005. Initial sequence of the chimpanzee genome and comparison with the human genome. Nature 437(7055):69-87.
6. Arnason U, Gullberg A, Janke A. 1998. Molecular timing of primate divergences as estimated by two nonprimate calibration points. J Mol Evol 47(6):718-27.
7. Sjøttem E, Anderssen S, Johansen T. 1996. The promoter activity of long terminal repeats of the HERV-H family of human retrovirus-like elements is critically dependent on Sp1 family proteins interacting with a GC/GT box located immediately 3′ to the TATA box. J Virol 70:188–98.
8. Pi W, Zhu X, Wu M, Wang Y, Fulzele S, Eroglu A, Ling J, Tuan D. 2010. Long-range function of an intergenic retrotransposon. Proc Natl Acad Sci USA 107:12992–12997.
9. Dunn CA, Romanish MT, Gutierrez LE. 2006. Transcription of two human genes from a bidirectional endogenous retrovirus promoter. Gene. 366(2):335-42.
10. Ting CN, Rosenberg MP, Snow CM. 1992. Endogenous retroviral sequences are required for tissue-specific expression of a human salivary amylase gene. Genes Dev 6(8):1457-65.
11. Cohen CJ, Lock WM, Mager DL. 2009. Endogenous retroviral LTRs as promoters for human genes: a critical assessment. Gene 448:105–114.
12. Muir A, Lever A, Moffett A. 2004. Expression and functions of human endogenous retroviruses in the placenta: an update. Placenta Suppl A:S16-25.
13. Villarreal LP. 2008. Origin of group identity: Viruses, addiction and cooperation.
14. Kamp C, Hirschmann P, Voss H. 2000. Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. Hum Mol Genet 9(17):2563-72.
15. Sutedja NA, Fischer K, Veldink JH. 2009. What we truly know about occupation as a risk factor for ALS: a critical and systematic review. Amyotroph Lateral Scler 10(5-6):295-301.
16. McCormick AL, Brown RH, Cudkowicz ME. 2008. Quantification of reverse transcriptase in ALS and elimination of a novel retroviral candidate. Neurology 70(4):278-83.
17. Li W, Lee MH, Henderson L, Tyagi R. 2015. Human endogenous retrovirus-K contributes to motor neuron disease. Sci Transl Med 7(307):1-12.
18. Maruszak H, Brew BJ, Giovannoni G, Gold J. 2011. Could antiretroviral drugs be effective in multiple sclerosis? A case report. Eur J Neurol 18:e110–1.
19. Cherkasova E, Weisman Q, Childs RW. 2013. Endogenous retroviruses as targets for antitumor immunity in renal cell cancer and other tumors. Front Oncol 3:243.
20. Van Der Kuyl AC. 2012. HIV infection and HERV expression: a review. Retrovirology 9:6.

21. Gold J, Goldacre R, Maruszak H, Giovannoni G, Yeates D, Goldacre M. 2014. HIV and lower risk of multiple sclerosis: beginning to unravel a mystery using a record-linked database study. J Neurol Neurosur PS jnnp–2014–307932.