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ENDOGENOUS RETROVIRUS SEQUENCES EXPRESSED IN MALE MAMMALIAN REPRODUCTIVE TISSUES: A REVIEW
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ENDOGENOUS RETROVIRUS SEQUENCES EXPRESSED IN MALE MAMMALIAN REPRODUCTIVE TISSUES: A REVIEW

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ABSTRACT

Objectives: **To review the research findings on the expression of endogenous retroviruses and retroviral-related particles in male mammalian reproductive tissues, and to discuss their possible role in normal cellular events and association with disease conditions in male reproductive tissues.**

Data sources: **Published findings on endogenous retrovirus (ERV) expression in vertebrate reproductive tissues.**

Study selection: **Relevant citations on ERVs and male reproduction by research groups worldwide.**

Data extraction: **Literature search on Medline and Pubmed upto the year 2000, and retrieval of relevant articles cited from international and local journals.**

Data synthesis: **Most of the studies demonstrated integrated retroviruses and retroviral-related sequences in human and mouse testis, epididymis and vas deferens. Endogenous retroviruses in human and mice may be associated with normal cellular differentiation and development, and carcinogenesis. In humans, one ERV family, human endogenous retrovirus-K (HERV-K) is abundantly expressed, and is associated with germ cell tumours, while ERV3 *env* is expressed in normal human testis.**

Conclusion: **The expression of ERVs in male reproductive tissues suggests a possible role in normal and disease conditions involving the testis and epididymis. These speculative functions may include among others spermatogenesis and or sperm maturation or tumour formation. However, further studies need to be carried out to investigate specific roles of ERVs in male reproductive events.**

INTRODUCTION

Endogenous retroviral (ERV) sequences comprise a significant proportion of the genome in most vertebrates and have been demonstrated in mice and primate reproductive tissues(1-5). Their presence in vertebrate genome dates back more than 30 million years(6). The origin of ERVs is not very clear, but most probably they are remnants of ancient germ line infections of exogenous retroviruses which subsequently got inserted in the host genome and have been transmitted as stable genetic traits(6,7). In principle ERVs have the same genomic organisation as exogenous retroviruses. Other investigators suggest that ERVs may have evolved from retroelements through transcription, retrotransposition and recombination events including addition of *env* genes(8). These retroelements may later become compatible and beneficial to the host. Approximately one to twelve per cent of the human genome consists of ERV elements(9). Their biological role is largely unknown despite the existence of many different families detected in the vertebrate genome. The majority of these viral sequences are defective due to presence of multiple termination codons and/or deletions, preventing translation to functional proteins(10,11). This may eventually result in either full-length or truncated proviruses, which are unable to code for infectious virus particles, with only a few of them coding for functional proteins(9). Although the genomic presence of most ERVs is presumed to be without a major effect, it is possible that some could be involved in normal or pathogenic processes(12). This is supported by studies in mice where a non-defective endogenous retrovirus has been demonstrated, which may induce tumours and/or immunological disorders(13,14).

The human retroviral elements analysed so far show sequence similarities to type A, B, C, and D murine and non-human primate retroviruses, as well as to human exogenous retroviruses(11,15). Since these retroviruses cover a range of structural variations that have been detected in infectious retroviruses such as HIV and SIV(10,16), they may contain a lot of viral information in the human genome. Because of such hypotheses, in this communication, we discuss the findings of various studies that have demonstrated expression of endogenous retrovirus-like particles in male mammalian reproductive tissues. In addition, we discuss the biological implications of the presence of these retroelements in these tissues. Because ERVs have been part of the genome of vertebrates for millions of years, it is likely that some of these sequences have acquired biological functions in the cell.

ENDOGENOUS RETROVIRAL GENOME ORGANISATION AND EXPRESSION

Endogenous retroviral sequences have the same fundamental structure as the integrated (proviral) form of an exogenous retrovirus in the host chromosomal DNA. Full-length ERVs possess three common retroviral genes, *gag, pol/prt* and *env*(7), which are located between two long terminal repeats (LTRs)(15). These genes code for the proteins essential for virus assembly, genome replication and integration, and enzymes essential for processing of viral proteins. Furthermore, the LTRs contain important signals needed for initiation, regulation, and termination of transcription(10). Solitary LTRs also occur in some cases(9,17). This probably originates from excision of the retroviral genome(17,18).

In normal cells the flow of genetic information usually involves transcription of DNA into RNA, which is later translated to specific proteins(19). As an exception to this flow of genetic information, in retroviruses RNA is copied into DNA(20). The viral RNA is first inserted into the cytoplasm of the host cell where it is utilised as a template in the synthesis of a proviral DNA catalysed by the enzyme reverse transcriptase (RNA-dependent DNA polymerase). Subsequently, this proviral DNA enters the nucleus where it integrates into the host DNA(12,21,22). In this way, retroviruses can colonise the host germline and inherited as endogenous retroviruses(23).

It has been suggested that deletions in retroviral genomes may occur at the RNA level by the reverse transcriptase skipping templates asymmetrically during virus replication(24). In turn, such deletion result in mutations that activate protooncogenes into transforming cellular genes(25).

ENDOGENOUS RETROVIRUS EXPRESSION IN MAMMALIAN MALE REPRODUCTIVE TISSUES

The human endogenous retrovirus-K (HERV-K) is the only known human endogenous virus genome that contains an open reading frame large enough to allow synthesis of full-length provirus, and is localised on chromosome-7(7,11). In line with this observation, complete open reading frames for all viral genes (*gag, pol, env)* have been observed(26). This retrovirus is present in about 30 to 50 copies in the human genome(18) with an estimated 10,000 solitary LTRs(17). In recent studies, sequence analysis of expressed HERV-K genomes revealed non-defective *gag* genes, a pre-requisite for particle formation. Open reading frames were also observed in *pol* and *env* genes. Antisera raised against recombinant *gag* proteins of HERV-K have been shown to stain human teratocarcinoma derived virus (HTDV) particles, linking them to the HERV-K family(27). Studies done in patients with gonadoblastomas and testicular biopsies, displayed HERV-K transcripts in neoplastic germ cells, and among the gonadoblastomas, an invasive seminoma was noted in two cases coupled with formation of additional germ cell tumour components(28). More so, expression of long terminal repeats has recently been demonstrated in both human and murine germ cell tumours(29), and these LTRs were shown to have dropped drastically after cellular differentiation(29). In another study, testicular germ cell tumours (TGCTs) of both adolescents and adults were investigated using reverse transcription-polymerase chain reaction. The *gag* and *prt* genes were expressed in all samples tested while *env* transcripts were detected in most normal testicular parenchyma samples. Simultaneous expression of multiple *gag* sequences was found both in normal parenchyma and TGCTs, and it was demonstrated that expression of gag sequences with an extra G, necessary to generate a functional protein, was not limited to TGCTs(4). Although the specific role of HERV-K expression remains unknown, such findings place HERV-K expression in an appropriate position for it to have a role in the pathogenesis of germ cell tumours. Similarly, differentiated human teratocarcinoma cell lines have been shown to produce the HTDV particles encoded by the human endogenous retroviral sequence HERV-K. According to Herbst and co-workers(28), among 2000 human sera samples screened against this endogenous human retrovirus, 60% of male patients with germ cell tumours demonstrated high titres. On the other hand, in healthy controls, anti-HTDV reactivity was only at low titres in a small percentage (3.9%) of the individuals tested. Additionally, HERV-K has been shown to carry responsive domains homologous to those of complex disease causing retroviruses such as HIV (Rev) and HTLV (Rex)(7,9). Rev viral element is a regulator of expression of viral proteins in HIV while Rex is it's equivalent in HTLV- 1. This has since strengthened the fact that it could be a biologically active ERV.

In a related study, 85% of patients with germ cell tumours were shown to produce antibodies against env *pro*tein of HERVs. From these individuals, those that received anti-tumour treatment showed a decrease in their antibody titre(30). HERV-K transcripts have been detected in all testicular germ cell tumours with the exception of teratomas and spermatocytic seminomas. More so, HERV-K expression has also been detected in testicular carcinomas *in situ* as well as gonocytes of dysgenetic gonad(3). Immunoblot analysis revealed an 80kDa HERV-K *gag* precursor and a 90kDa viral env protein(31). The expression of HERV-K in human teratocarcinoma cell lines was related to higher levels of ß-human chorionic gonadotropin (ß-HCG) synthesis(32).

In other studies HERV-K like expression has also been demonstrated in disease-free adult mice testes, and was found restricted to undifferentiated spermatocytes(31). Another ERV family expressed in male reproductive tissues is ERV3 (HERV-R). ERV3 *env* mRNA has been highly demonstrated in spermatogenic cells in human testes during early phases of spermatogenesis but could not be identified in sertoli or leydig cells(33). In addition, sperm adsorbed with retrovirus-like particles have been detected in mice epididymal spermatozoa(2). Viral

aggregates evident in the epididymal lumen leads to the hypothesis that the lumen of the epididymis is the principal site of endogenous virus synthesis(2). In a related study, retroviral reverse transcriptase activity was detected in epididymal fluids from several strains of mice(34) signifying the expression of pol genes. Similarly, Kiessling and co-workers(34) have indirectly shown the presence of proteins from the retrovirus gag gene product (p30) and *env* gene product (gp70) through immunoblot analysis of epididymal fluids from four mouse strains. The expression was found to be consistent for several groups of males tested over a period of fifteen months indicating that the viral expression was not due to exogenous infection. These results suggest that the presence of epididymal viruses may represent specific transcription of endogenous retroviral genes in the reproductive tract. In addition, it gave evidence that reverse transcriptase activity is ubiquitous in the male reproductive tract of mice and may or may not be associated with virus particles(1). A high incidence of spontanous virus expression has also been demonstrated in New Zealand Black mice, where the reproductive tract of males were shown to contain C-type retrovirus(35). Also endogenous mouse mammary tumour virus (MMTV) proteins (p28 and gp47) have been identified in the epididymis and seminal vesicles of adult Swiss Albino mice devoid of exogenous virus(13).

It is also important to note that the expression of ERVs seems to be steroid-dependent. This is demonstrated by the presence of ERV mRNA in normal and pathological conditions that result in steroid hormone synthesis. For instance, a report from studies done on normal human skin and dermoid cysts of the ovary, ERV3 *env* mRNA expression was demonstrated in sebaceous glands by *in situ* hybridisation(36). Since it is known that the regulation of sebaceous glands is primarily via steroid hormones, particularly androgens, it is possible that expression of ERVs is hormone dependent. This hypothesis is backed by specific expression of ERVs in reproductive tissues, these are active sites for steroid hormones synthesis and action. Our own work have demonstrated ERV3 *env*-like particles in adult male baboon testes and also on spermatozoa within the caput epididymis. In addition, the epithelia of cauda epididymis showed the expression of the same antigen, and reverse transcriptase activity was detected in semen and spermatozoa from ejaculum and epididymis (Sichangi *et al*, manuscript in preparation). This work was the first of its kind to be studied in male non-human primates.

POTENTIAL ROLES OF ENDOGENOUS RETROVIRUSES IN MALE REPRODUCTIVE TISSUES

The origin of virus-like particles (endogenous) as well as their role in normal and in the pathogenesis of germ cell tumours in the male is not known as yet. It is proposed that their presence in reproductive tissues may induce a variety of interactions with the host cells and exogenous factors that may lead to multiple consequences on the host. Due to such possible interactions, several roles have been postulated for ERVs both in normal and disease conditions involving male reproductive tissues. The elevation of ß-hCG synthesis and HERV-K serum antibody reactivity in teratocarcinoma patients have been demonstrated(32). This association may implicate HERV-K in the pathogenesis of teratocarcinomas and other germ cell tumours. The association of HERV-K reverse transcriptase with germ cell tumours may suggest a role in the pathogenesis of such tumour(29). These may produce germ line mutations(37) resulting in loss of gene function that may be important, including tumour suppresser genes(38). Loss of such genes lead to germ cell tumour formation. Endogenous retroviruses have been implicated to act as somatic-to-germ line genetic vectors, whereby somatically expressed genes can be returned to the germ line. The mechanism so far described proposes that these interactions occur in the epididymis of the male reproductive tract and are restricted to memory lymphocytes(39).

The presence of endogenous virus in male reproductive tract as well as developing spermatogenic cells(33,34) is consistent with the suggestion that the viruses have a normal biological role. Such circumstantial evidence does not, however, indicate whether viral expression only accompanies, or does influence, cell differentiation. But the fact that retroviral-like particles have been sustained within the germ line, and the way they have been distributed among species strongly suggests a possible role in the biology of their hosts. The expression of ERV 3 *env* elements in human(33) and baboon (Sichangi *et al*, unpublished data) undifferentiated spermatogenic cells indicates a possible role in germ cell development(29,33). Endogenous retrovirus 3 *env* protein has been shown to carry a domain that shares homology with p15E. Protein 15E has been demonstrated to exhibit both immunosuppressive and fusogenic properties(40). This fusogenic property is further supported by recent studies in which HERV-W, a new family of human endogenous retrovirus was localised in human testis, and subsequently found to express syncytin, a protein demonstrating fusogenic properties(5). This property may be of relevance in enhancing fusion of spermatozoa expressing this protein with the zona pellucida and/or oocyte membrane during fertilisation. However, this is a speculation that cannot be concluded at this level of discussion. Endogenous retroviral association with germ cells may also result in genomic plasticity through rearrangements caused by recombination events(8).

CONCLUSION

Additional information on the presence of virus particles expressed in male reproductive tract, and the site of synthesis of the reverse transcriptase-like enzyme activity in semen and epididymal fluid is necessary to understand their role in male reproductive activities. There is accruing

evidence that some ERVs families could be having a biological role associated with spermatogenesis. This is based on the demonstration of ERV3 *env* mRNA in human testes, and intracisternal A- type particles in mice. All these were localised in early phases of spermatogenesis. Our studies have shown ERV3 *env* proteins in early phases of spermatogenic cells of mature baboon testes and in epididymides. A pathological role in germ cell tumour formation has also been postulated, and more strength on this is added by the association of HERV-K family with such tumours. The level of action seems to be at cellular differentiation since ERV expression is high in actively differentiating cells. However, there is still need for further studies into this interesting phenomena associated with these foreign agents that have become part of human genome.

REFERENCES

1. Kiessling, A. A., Crowell, R. and O'Connell R. S. Sperm-associated retroviruses in the mouse epididymis. *Proc. Natl. Acad. Sc. (USA),* 1987; **84**:8667-8671.
2. Kiessling, A. A, Crowell, R. and Fox, C. Epididymis is the principal site of retroviral expression in the mouse. *Proc. Natl. Acad. Sc. (USA).* 1989; **86**:5109-5114.
3. Herbst, H., Sauter, M. and Mueller-Lantzsch, N. Expression of human endogenous retrovirus K elements in germ Cell and trophoblastic tumours. *Amer. J. Pathol.,* 1996; **149**:1727-1735.
4. Roelofs, H., Ruud, J. H. L. M. van Gurp., Oosterhuis, J. W. and Looijenga, L. H. J. Detection of Human Endogenous Retrovirus Type K-specific Transcripts in Testicular Parenchyma and Testicular Germ Cell Tumours of Adolescents and Adults. *Amer. J. Pathol.* 1998; **153**:1277-1282.
5. Mi, S., Lee, X., Li, X., Vedrnan, G. M., Finnerty, H., Racie, L., Lavallie, E., Tang, X., Edouard, P., Howes, S., Keith, J. C. and Mc Coy, J. M. Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature.* 2000; **40**:785-789.
6. Benveniste, R. E. and Todaro, G. J. Evolution of type-C viral genes: evidence for an Asian origin of man. *Nature.* 1976; **261**:101-109.
7. Magin, C., Lower, R. and Lower, .T. cORF and RcRE, the Rev/Rex and RRE/RxRE homologous of the human endogenous retrovirus family HTDV/HERV-K. *J. Virol.* 1999; **73**:9496-9507.
8. Taruscio, T. and Mantovani, A Human endogenous retroviral sequences: Possible roles in reproductive physiopathology. *Biol. Reprod. 1998;* **59**:713-724.
9. Knossl, M., Lower, R. and Lower, J. Expression of the human endogenous retrovirus HTDV/HERV-K is enhanced by cellular transcription factor YYl. *J. Virol.* 1999; **73**:1254- 1261.
10. Larsson, E., Kato, N. and Cohen, M. Human endogenous proviruses. *Curr. Top. Microbiol. Immunol.* 1989; **148**:115-132.
11. Leib-Mosch, C., Brack-Wemer, R., Wemer, T., Bachmann, M., Faff, O., Erfle, V. and Hehlmann, R. Endogenous retroviral elements in human DNA. *Can. Res. (Suppl.),* 1990; **50**:5636s-5650s.
12. Lower, R., Lower, J. and Kurth, R. The viruses in all of us: Characteristics and biological significance of human endogenous retrovirus sequences. *Proc. Natl. Acad. Sc. (USA),* 1996; 93:5177-5184.
13. Osterrieth, P.M., Kozma, S., Hendrick, J. C., Francois, C., Calberg-Bacq C. M., Franchimont, P. et al. Detection of virus antigens in Swiss albino mice infected by milkborne mouse mammary tumour virus: the effect of age, sex and reproductive status. II Radioimmunoassay of two virus components, gp47 and p28 in serum and organ extracts. *J. Gen. Virol,* 1979; **45**:41-50.
14. Marrack, P., Kushmir, E. and Kappler, J. A maternally inherited superantigen encoded by a mammary tumour virus. *Nature.* 1991; **349**:524-526.
15. Doolittle, R. F., Feng, D. F., Mc Clure, M. A. and Johnson, M. S. Retrovirus phylogeny and evolution. *Curr. Top. Microbiol. Immunol.* 1990; 157:3-4.
16. Wilkinson, D.A., Mager, D. L. and Leong, J. C. Endogenous human rekoviruses. In: Levy, J. (ed). Retroviridae, Plenum press, volume III. New York, N. Y.
17. Leib Mosch, C., Haltmeier, M., Werner, T., Geigl, E., Brack-Wemer, R. and Francke, U. et al. Genomic distribution and transcription of solitary HERV—K LTRs. *Genomics.* 1993; 18:261-269.
18. Ono, M., Yasunaga, T., Miyata, T. and Ushikubo, H. Nucleotide sequence of human endogenous retrovirus genome related to the mouse mammary tumour virus genome. J. *Virol.* 1986; **60**:589-598.
19. De Robertis, E. D. P. and De Robertis, Jr. E. M. F. Nucleicacids. In, De Robertis, E. D. P. and De Robertis, Jr. E. M. F. (eds). Cell and molecular biology, eighth edition. Pp 29.
20. Lewin, R. How mammalian RNA returns to its genome. Work with certain small nuclear RNAs is giving clues about the apparently common flow of information from RNA back to DNA. Science, **219**:1052- 1 054.
21. Watson, J. D., Tooze, J. and Kurtz, D. T. The RNA tumour viruses replicate by means of a double-stranded DNA intermediate. In: Recombinant DNA: A Short Course, Scientific American Inc. 1983. Pp. 54.
22. Huang, L., Silberman, J., Rothschild, H. and Cohen, J. C. Replication of endogenous virus in human cells (kinetics of DNA synthesis and integration). J. Biol. *Chem.* 1989; **261**:8811-8814.
23. Stoye, J. P. and Coffin, J. M. A provirus put to work. *Nature,* 2000; **403**:715-717
24. Coffin, J. M. Structure, replication and recombination of retrovirus genomes: Some unifying hypothesis. J. *Gen. Virol.* 1979; **421**:1-26.
25. Coffin, J.M. and Rosenberg, N. Retroviruses. *Nature,* 1999; **399**:413-415.
26. Mayer, J., Sauter, M., Racz, A.. Sherer, D., Mueller-Lantzsch, N. and Meese, E. An almost intact human endogenous retrovirus-K on human chromosome 7. *Nat. Gen.* 1999; **21**:257-258.
27. Lower, R., Boller, K., Hasenmaier, B., Korbmacher, C., Muller-Lantzsch, N. and Lower, J. et al. Identification of human endogenous retroviruses with complex mRNA expression and particle formation. *Proc. Natl. Acad. Sc., USA.* 1993; **90**:4480-4484.
28. Herbst, H., Kuhler-Obbarius, C., Lauke, H., Sauter, M., Mueller-Lartzsch, N., Harms, D. et al. Human endogenous retrovirus (HERV)-K transcripts in gonadoblastomas and gonadoblastoma-derived germ cell tumours. *Virc. Arch. 1999;* **434**:5-11.
29. Casau, A. E., Vaughan, J. E., Lozano, G. and Levine, A. J. Germ cell expression of isolated human endogenous retroviral long terminal repeat of the HERV-K HTDV family in transgenic mice. *J. Virol.* 1999; **73**:9976-9983.
30. Sauter, M., Roemer, K., Best, B., Afting, M., Schommer, S., Seitz, G., Hartmann, M. and Mueller-Lantzsch, N. Specificity of antibodies directed against env protein of human endogenous reroviruses in patients with genn cell tumours. *Can. Pes.* 1996; **56**:4362-4365.
31. Boller, K., Janssen, O., Schuldes, H., Tonjes, R. R., Kurth, R. Characterisation of the antibody response specific for the human endogenous retrovirus HTDV/HERV-K. *J. Virol.,* 1997; **71**:4581-4588.
32. Lower, R., Lower, J., Frank, H., Harzmann, R. and Kurth, R. Human teratocarcinomas cultured in vitro produce unique retrovirus-like viruses. *J. Gen. Virol.* 1984; **65**:887-898.
33. Larsson, E., Anderson, A. and Nilsson, O. Expression of endogenous retrovirus ERV3 (HERV-R) in human reproductive and embryonic tissues-evidence for a function for envelope gene products. *Upps. J. Med. Sc.,* 1994; **99**:113-120.
34. Kiessling, A. A. Evidence that reverse transcriptase is a component of murine epididymal fluid (41859). *Proc. Soc. Exp. Biol. Med.* 1984; **176**:175-182.
35. Femandes, G., Yunis, E. and Good, R. A. Reproductive deficiency of New Zealand black male mice: Possibility of a viral basis. *Lab. Inv.* 1973; **29**:278.
36. Andersson, A. C., Merza, M.. Venables, P., Ponten, F., Sundstrom, J. and Cohen, M. et al. Elevated levels of the endogenous retrovirus ERV3 in human sebaceous glands. *J. Inv. Dermat. 1996;* **106**:125-128.
37. Morse, B., Rotherg, P., Spandorfer, J. and Astrin, S. Insertion mutagenesis of the myc locus by a LINE-I sequence in a human breast carcinoma. *Nature,* **333**:87-90.
38. Miki, Y., Nishisho, I., Horii, A., Miyoshi, Y., Utsonomiya, J. Kinzler, K. *et al.* Disruption of the APC gene by a retrotransposal insertion of Ll sequence in a colon cancer. *Can. Resear.* **52**:643-645.
39. Rothenfluh, H. S. Hypothesis: a memory lymphocyte specific soma-to-germ line genetic feedback loop. *Immunol. Cell Biol.* 1995; **73**:174-180.
40. Lin, L., Xu, B. and Rote, N. S. Expression of endogenous retrovirus (ERV-3) induces differentiation in BeWo, a choriocarcinoma model of human placental trophoblast. *Placenta.* 1999; **20**:109-118.