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EVIDENCE FOR EXPRESSION OF ENDOGENOUS RETROVIRAL SEQUENCES ON PRIMATE REPRODUCTIVE TISSUES AND DETECTION OF CROSS-REACTIVE ERVS ANTIGENS IN THE BABOON OVARY: A REVIEW

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ABSTRACT

Objective: **To review recent research findings on the specific expression of endogenous retroviral sequences (ERVS) in reproductive tissues and their possible physiological roles. ERVS have been implicated in several biological events such as induction of resistance to exogenous retrovirus invasion, involvement in placental trophoblast formation, sperm maturation and differentiation; and stimulation of local immunosuppression to protect the foetus from immunological attack.**
Data sources: **Critical review of relevant articles and abstracts cited in international and local journals, literature searches on Medline and Medchem up to 2005.**
Data synthesis: **Retroviruses have been implicated in the induction of tumour and immunological disorders. Over the years, endogenous retroviruses (ERVs) and retroviral elements have been detected in the genome of many vertebrate species, including primates. The evidence for the presence of retroviruses in the primate tissues such as the placenta, ovary, breast, testis and epididymis has been documented using electron microscopic studies. Retrovirus-like particles were found budding from the basal membrane of syncytiotrophoblasts, as well as in tumour cell lines in embryonic carcinoma or teratocarcinomas. Apart from their pathological effects, recent evidence suggests that these ERVs may play useful roles in normal physiological events.**
Results: **Recent studies indicate the expression of endogenous retroviruses in the testis, epididymis, placenta and breast. However, limited data exist on the detection of ERVs in the ovary. Overall, the precise functions for ERVs in these tissues are not well understood. In the testis and epididymis, speculative functions may include among others spermatogenesis and/or sperm maturation (differentiation) whereas in placenta they are possibly associated with trophoblast fusion and locally induced immunosuppression to protect the foetus from immunological attack. Experiments in our laboratory have indicated restricted expression of retroviral antigens including baboon endogenous retroviral proteins (BERV), ERV-3, HIV-1 gp41 and HERV-K env in the baboon ovary.**
Conclusion: **ERVs are specifically expressed in different mammalian reproductive tissues and may have unique physiological roles.**

INTRODUCTION

Retroviruses are a diverse group of Ribonucleic Acid (RNA) viruses that can transcribe RNA to Deoxyribonucleic Acid (DNA) by use of reverse transcriptase and subsequently integrate the viral DNA as a provirus into the DNA of the host cell. Retroviruses belong to the retroviridae family, which is sub-divided into three major sub-families based on the apparent consequences of infection namely oncovirinae, lentiviridae and spumavirinae (1).

Also, typical retroviruses have been classified either as endogenous or exogenous viruses on the basis of integration into somatic cells, latency period and mode of transmission (2,3). In this review, we discuss the features of ERV and their expression in female and male reproductive tissues such as placenta, ovary, breast, testis and epididymis and their possible physiological roles.

Features of endogenous retroviruses

Endogenous retroviruses (ERVs) are viral elements carried vertically in the germ line and comprise a significant proportion (up to 5%) of most vertebrate genomes (3). They have been classified on the basis of morphological characteristics observed with the electron microscope as types A, B, C, D (4). These particles have structural homologies to exogenous retroviruses and are commonly expressed in reproductive tissues (5). In contrast to exogenous retroviruses, endogenous retroviruses are thought to originate from previous retroviruses that may have entered the germ cells of vertebrates (6). The vast majority of ERV loci are devoid of gene expression due to random mutations. The basic retroviral genome organization consists of a linear array of genes that can be sub-divided into *gag, pol* and *env* as well as 3′ and 5′ long terminal repeats (LTR) regions.

Alternatively ERVs may have evolved from ancestral elements through transposition and recombination, including the addition of *env* genes (2). Intact genomes of baboon endogenous virus (BaEV) are present in the *papionini* monkeys (except macaques) and in four species of African green monkey *(Cercopithecus aethiops)* (7,8). Mammals appear to have retained the presence of at least some copies of non-defective genomic retroviruses, such as intracytoplasmic A-type particles (IAPs) and type C endogenous retroviruses (6,9–12). It is difficult to explain the selective pressure that maintains these ERVs in the genome. Endogenous retroviruses have been implicated as causative agents of diseases notably neoplasia and autoimmune diseases (1,13). However, since these viruses have persisted in the cells of organisms, it is possible that they have a physiological significance to the host.

Research is on-going to determine their physiological roles on the host especially in the reproductive tissues where they are abundantly expressed.

Expression of ERV in reproductive tissues

ERV-3 gene products are expressed mostly in normal human placental tissues but are absent from trophoblastic tumours such as choriocarcinoma (5). Approximately, 5% of the human genome consists of human endogenous retrovirus (HERV) sequences (14). *In situ* hybridization studies have shown that ERV-3 *env* is expressed not only in the placenta but also in other human reproductive tissues such as testis, breast, embryonic tissues and some tumours (Table 1) (5,15). Another human HERV, HERV-W, is mostly expressed in placental cells (16). Although most HERVs are frequently expressed in placenta and to a less extent in other tissues (6,14), few HERVs express complete genome including envelope (*env*). The selective pressure potentially exerted by evolution to maintain some HERV *env* open reading frames (ORE) and restrict their expression in specific tissues, suggests that these ERVs may exhibit a positive role, provided adequate control mechanisms and regulation of expression exist in the host (17). Indeed the positive role for the *env* has been suggested such as inducing resistance to exogenous retrovirus invasion by receptor interference, conferring local immunosuppression or by mediating placenta syncytium formation (5,6,18). Expression of other retroviral-like proteins and mRNA transcripts, such as HERV-K, have also been demonstrated in normal adult mice testis and was found restricted to the undifferentiated spermatocytes as well as in normal human tissues (19,20). Budding type C and intracytoplasmic type A particles IAPs have been detected in Chinese hamster ovary cells (21) but infectivity of these retroviral particles has not been demonstrated (21).

Table 1

Occurrence of ERVs and their postulated physiological roles

ERV	Main tissues of occurrence	Postulated physiological role
ERV-3	Placenta tissues (5) Testis, epidydimis (5) Breast tissues (23)	Trophoblast and syncytium formation (5,22) Sperm maturation and/or differentiation (5) Immunosupression and breast cancer (23)
HERV-W	Mostly placental tissues (6,14,16)	Trophoblast formation (6,14,16) Local immunosupression to protect the foetus from immunological attack (6,18)
HERV-K	Testis (19,20)	Sperm maturation and/or differentiation (19,20)
BaERV	Genome (1,13)	Neoplasia and autoimmune diseases (1,13)
Budding type-C and IAPs	Ovary (21)	Interact with steroid hormones (21)

Expression and the role of ERVs in placenta

It has been known for sometime that viral envelope *(env)* proteins, have properties that enable the virus to bind to a specific receptor on target cell, fuse with the cell and mediate viral entry. This property could mediate trophoblast fusion (26). Indeed in the placental tissue, ERV-3 envelope glycoproteins are abundantly expressed and have been shown to participate in syncytiotrophoblast differentiation by fusion of the underlying cytotrophoblast cell layer (27). This fusion process is associated with the expression of several types of endogenous retroviral particles in the placenta (27). It is possible that ERV-3 envelope glycoprotein can play different roles, such as inducing differentiation of the cytotrophoblastic cells (28), also preventing maternal immune rejection of the foetus (27). Studies by Mi *et al* (29) suggested that ERV protein (syncytin) might be essential for placental development and differentiation. Notably, syncytin was found to be identical to the *env* gene product of HERV-W (16,29). Syncytin is expressed at high levels in the human placenta, especially in the syncytiotrophoblast layer (27). Apparently, syncytin appears to mediate the fusion of cytotrophoblast cells to form syncytiotrophoblast layer. Expression of ERV-related antigens was demonstrated in the baboon placenta (30) and it is possible similar events occur during placental development in the baboon and other non-human primates.

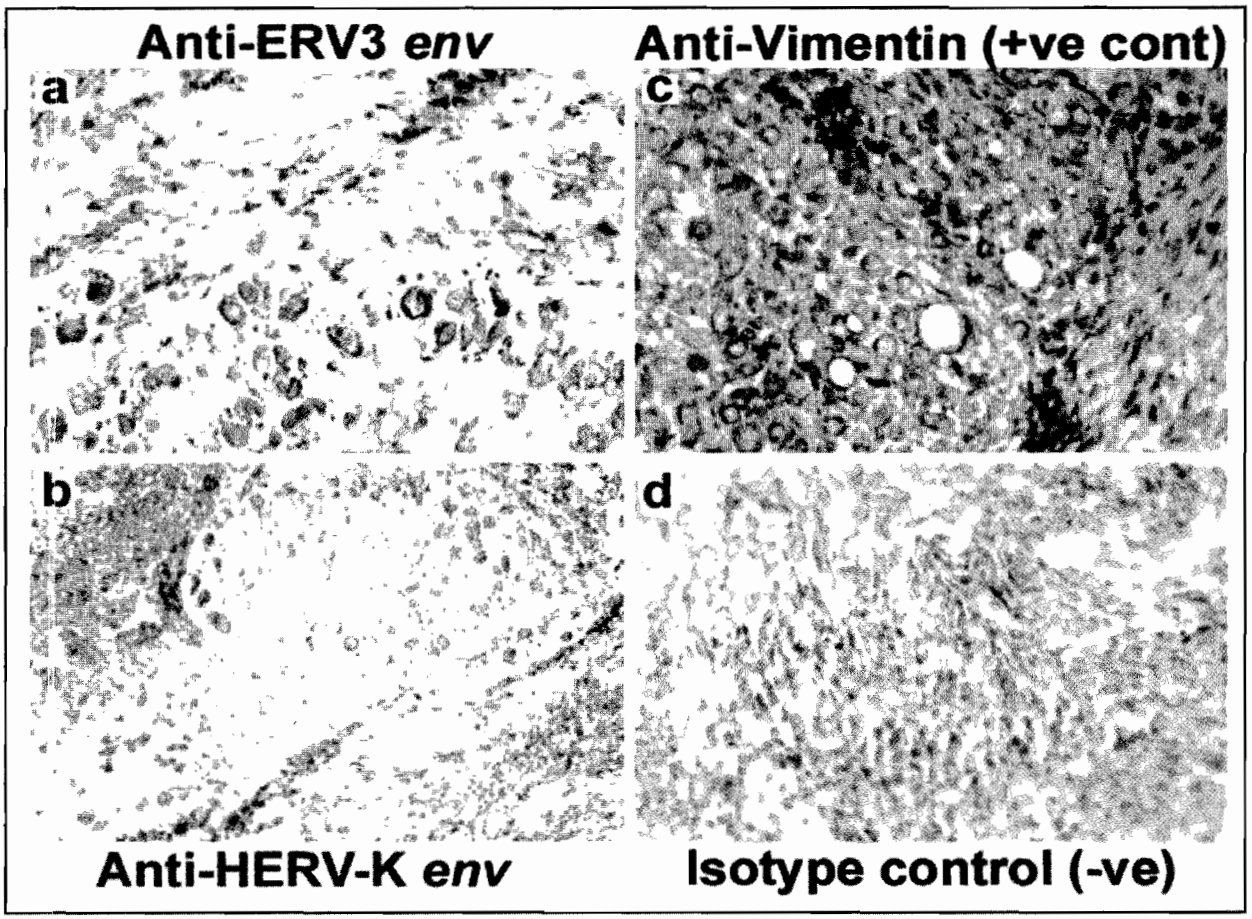
Expression of ERV in the ovary and ovarian tumours

Studies have also revealed the expression of ERV in human oocytes and follicular fluids which cause ovarian tumours (31). It became apparent from the literature review that limited studies have focused on ERV expression in primate ovary. Thus, we initiated studies to determine the expression of endogenous retroviral antigens on the baboon ovary. In this study, we stained both fresh-frozen and formalin-fixed baboon ovarian tissues with different antibodies raised against human and non-human primate endogenous and exogenous viruses (BERV, SIV, HIV-l, HIV-2 and HERV, (Table 2), using the Streptoavidin-Biotin peroxidase method (Histostain-SP kit, Zymed Laboratories, San Francisco, CA).

The same antibodies had been used in previous studies (32). In this study, we have shown that antibodies against the following retroviral proteins, ERV-3, HIV-l gp 41, HERV-K env and BERV were reactive with the baboon ovarian tissues (Table 2 and Figure 1). Overall, this pilot study indicates specific expression of retroviral-related antigens in the baboon ovary. Other investigators have shown the presence of budding type C and intracytoplasmic type A particles in Chinese hamster ovary cells (21). However extensive screening failed to detect any evidence of infectivity of these retroviral particles (21).

One striking feature of type C retroviruses is their apparent hormonal responsiveness and the

Figure 1



Immunohistochemical localization of endogenous retroviral proteins in normal baboon ovary. The sections were stained with the following monoclonal antibodies: anti-ERV3 env (a), anti-HERV-K env (b), anti-Vimentin (positive control, c) and isotype-matched control antibody (d). Positive reactivity is shown by the red precipitate. a) ERV3 env protein was highly expressed by the granulosa cells in the normal baboon ovary. The protein was mainly expressed in the cytoplasm. b). The granulosa cells also showed strong positive staining for HERV-K env protein. c). Anti-vimentin staining was used as an experimental positive control and stained intracellular vimentin in the ovary as expected. (d) Negative controls included isotype-specific antibodies and substituting PBS for primary antibody. As expected, none of the tissues stained positive. Mag. x200.

possible involvement of steroid hormones in regulating the functions of ERVs (24, 25). The detection of these ERV-related antigens in the baboon ovary as reported in the current study also supports the possible role of steroid hormones in regulating the expression of ERV in the ovarian endocrine micro-environment.

Expression and the role of ERV in breast and involvement in tumours and carcinomas

It would appear that retroviruses suppress the immune system through their gene product (p15E) and this may lead to development of malignancy such as breast cancer (15). Early reports presented evidence that p15E, a gene product of ERV, may be associated with human breast cancers (23). It is thought that this protein down regulates immune responses and prevents killing of cancerous cells by the immune cells (33). Evidence is provided by experiments showing p15E-like proteins in serum, urine and tumour effusions of cancer patients suppress immune responses that can be reversed by anti-p15E antibody (23). However, the involvement of ERV-3 *env* in protecting trophoblast from destruction by immune cells remains unclear since a stop codon occurs in the membrane domain of ERV-3 *env* gene (34) and since recent studies have

Table 2

Immunohistochemical reactivity of mouse anti-ERV antibodies with baboon ovary tissue

Antibody	Reactivity
Anti-Vimentin (+ ve control)	++
W6/32 (+ ve control)	++
PBS (-ve control)	–
BERV 13	++
BERV 14	++
BERV 15	++
BERV 16	–
Mab to SIV P27	–
Mab to HIV-2 gp 120	–
Anti-SIV Mac P17	–
Anti-HERV-K RT	–
Anti- ERV3 *env*	++
Anti-HIV-1 P24/25 *gag*	+/–
Mab to HIV-1 gp 41	++
Anti-HERV-K *env*	+/–
Anti-HERV-K *env*	++
MAB to SIV Mac 251 *gag*	+/–

– Negative
± Weak positive
++ Strong positive
BERV Polyclonal antibody against crude baboon endogenous retrovirus antigen
W6/32 Monoclonal antibody against human MHC class I determinants
PBS Phosphate buffered saline

showed a mutation in the ERV-3 *env* gene in about 1% of homozygous individuals (35). HERV-W that expresses a complete *env* gene (36) has also been described and proposed to promote cell fusion and may have immunosuppressive properties. However, the absence of HERV-W sequences in New World monkeys (37) indicates that local immune suppression may be induced by distinct and/or complementary mechanisms.

Expression of ERV in male reproductive tissues

In normal human testis ERV-3 *env* showed a pattern of expression similar to that of IAPs that is restricted to the first phases of spermatogenesis and was not identified in Sertoli or Leydig cells (5). Expression of other retroviral-like proteins and mRNA transcripts, such as HERV-K, have also been demonstrated in normal adult mice testis and was found restricted to the undifferentiated spermatocytes as well as in normal human tissues (19,20). ERV related antigens have been described in the baboon reproductive tissues including testis (38, 39). In the testis, it has been suggested that ERV could play a role in spermatogenesis and or sperm maturation (differentiation) or tumour formation (5). Recently, HERV-K transcripts have also been detected in gonadoblastomas and gonadoblastoma-derived germ cell tumours as well as in testicular parachyma and testicular germ cell tumours of adolescents and adults (40,41). Transcripts encoding the envelop region, HERV-E have also been found expessed in human prostate carcinoma (42).

CONCLUSION

Restricted tissue specific expression of ERVs in various reproductive tissues such as the placenta, testis and ovary supports the potential physiological roles of ERVS. Such roles include placental trophoblast formation and sperm maturation and differentiation. The expression of ERV in baboon ovary (BERV) may indicate interaction between ERV and hormones and hence possible involvement of ERV in cellular differentiation in the ovary.

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