Upsala J Med Sci 99: 113–120, 1994

Expression of an Endogenous Retrovirus (ERV3 HERV-R) in Human Reproductive and Embryonic Tissues—Evidence for a Function for Envelope Gene Products

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ABSTRACT

ERV3 (HERV-R) is a complete human endogenous retrovirus located on the long arm of chromosome 7. It is expressed in several human tissues as LTR env spliced transcripts (9 and 3.5 kb). The highest level of expression is to be found in placenta and virus expression is down-regulated in choriocarcinoma cell lines. By means of in situ hybridization, the expression of ERV3 env was studied in selected human reproductive and embryonic tissues. It is concluded that (a) ERV3 env is expressed in syncytiotrophoblasts not only in the placenta but also in hydatidiform moles and choriocarcinomas (irrespective of origin) (b) ERV3 expression in placenta correlates to cell fusion but probably not to the fertilization process itself (c) ERV3 env is highly expressed in certain cells in spermatogenesis but not in the Sertoli or Leydig cells, and finally (d) ERV3 env is expressed in certain embryonic tissues such as the adrenal gland and nervous tissues.

INTRODUCTION

The presence of retroviral and retroviral-like particles in different reproductive tissues, oocytes and preimplantation embryos has puzzled scientists for some time. There have been claims that a specific expression of retroviruses exists during implantation and early embryogenesis (for early review see 18 and 4). An attractive hypothesis is that endogenous retroviruses (ERV) could play a role in these early events. The expression of retroviruses and/or retroviral related sequences in humans has been detected in reproductive tissues particulary the placenta (9, 12, 17). The hypothesis that ERVs are involved in specific processes during early embryogenesis has gained new strength since it is now clear that human DNA, like the DNA of other species, contains several

integrated retroviruses and retroviralrelated sequences. (For reviews see 9, 12 and 17). There is also a growing body of evidence that ERVs could be involved not only in important normal cellular processes like differentiation and specialization but also in tumorigenesis and immunomodulation(6), the latter as a consequence of a defective cellular control of ERV expression. Nevertheless, correlation between the expression of ERV and any biological function is yet to be discovered. As a first step towards an understanding of the role of ERVs, the expression of one ERV, i.e. ERV3 was studied in human tissues.
ERV3 is a complete human ERV located on the long arm of chromosome 7. This retroviral genome contains an open reading frame (ORF) throughout the env gene but has in frame termination codons in the gag pol genes, which should preclude the expression of the virus as particles but may produce typical retroviral proteins especially the env encoded p15E and gp70 (14). We have previously shown, by Northern blot analysis, that ERV3 env is readily expressed in most human tissues except choriocarcinomas (6). In this communication we have found, by in situ hybridization, that ERV3 env is expressed not only in the placenta but also in other human reproductive tissues such as the testis, embryonic tissues and trophoblastic tumours of ovarian origin.

MATERIALS AND METHODS

The following human tissues were used for in situ hybridization (ISH):
Normal placenta throughout a complete gestational period
Normal endometrium
Endometrium of pregnancy, decidua and placental tissues from the same patient
Hydatidiforme mole (two cases)
Endometrial choriocarcinoma (two cases)
Ovarian choriocarcinoma (two cases)
Normal testis (one case)
Testis with Seminoma and testis with Choriocarcinomas (two cases each)

Normal and neoplastic human tissues were obtained from the department of Pathology, University Hospital Uppsala. Representative sample sections were fixed, embedded in paraffin and processed for routine histology. The normal fetal material was obtained from patients operated for ectopic pregnancies, where normal fetal material was also observed.

IN-SITU HYBRIDIZATION (ISH)

ISH was performed as described previously(5). Briefly, paraffin embedded tissues were sectioned 4μm thick and mounted on 3-aminopropyltrietoxylsilane-coated slides (Sigma). Sections were pre-treated with 0.2 M HCl for ten minutes and permeabilized with 2ug/ml Proteinase-K (Merck) at 37°C for 15 min prior to hybridization. Tissue sections were hybridized with 35-S labelled riboprobes (Promega Biotech) transcribed from supercoiled plasmid templates, yielding probes of consistent activity and size.
Subsequently they were hybridized overnight at 56° C in 3x standard saline extract and 50% formamide and washed in 2x standard saline citrate and 50% formamide prior to treatment with RNase A (Boehringer), (100ug/ml 37°C for 30 min). The application of NT2B photographic emulsion (KODAK) diluted 1:1 in distilled water was followed by exposure at 4°C for 2-4 weeks. Slides were developed and counterstained with Mayers hematoxylin and mounted with Permount (Fischer Scientific). The 1.75 kb ERV3 env riboprobe transcribed in both directions was used as probes (6). The sense probe was used as negative control.

RESULTS

We have previously stated that ERV3 env is expressed in most human tissues especially the placenta(6). These data were obtained by Northern blot analysis of RNA extracted from whole tissue samples. In this study we have localized the viral expression in individual cells by ISH.

PLACENTAL TISSUES

ERV3 env expression in the placenta is localised, almost exclusively, to the syncytiotrophoblasts throughout pregnancy. Expression is thus localized only to cells undergoing fusion (Fig1). Proliferating cytotrophoblasts were either negative or expressed very small amounts of the env gene as did the cells of the intervillous stroma. A sense probe, included as a control, remained negative in the ISH assay. Decidual and endometrial glands expressed low levels of ERV3 transcript.

HYDATIDIFORM MOLES AND CHORIOCARCINOMAS

Hydatidiform moles contain syncytiotrophoblastic cells to a certain extent. However, the syncytiotrophoblasts in these tissues seem to express ERV3 env with the same degree as normal placenta.

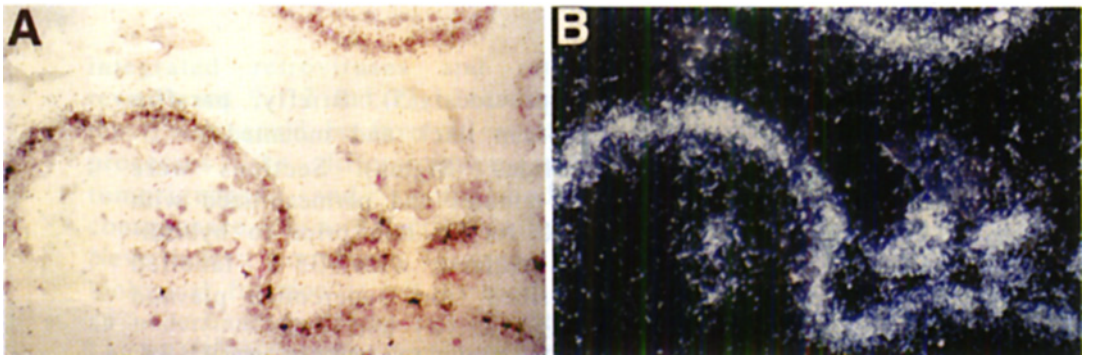

Fig1. Localization of ERV3 env in term placenta by in situ hybridization. The hybridization product is exclusively localized to the cyto - syncytiotrophoblastic cell layer and in particular to the syncytiotrophoblastic cells (Left photo bright field and right dark field.)

Choriocarcinomas from different locations were included in this study. These included both endometrial and ovarian choriocarcinomas obtained from elderly women to exclude the possibility of ectopic pregnancies. Ovarian choriocarcinomas is extremely rare and we could find only two cases registred in our pathology files during the last decade. Two cases of testicular choriocarcinomas were also investigated. The majority of cells in all these cases comprised of cytotrophoblasts but some multinucleated syncytiotrophoblasts were also seen, so considered because of their HCG expression demonstrated immunocytochemically. In all cases, ERV3 env expression was detected either in fused cells or weak and scattered in the cytotrophoblasts. The intensity of the hybridization signal in the cells that expressed ERV3 env was the same as in the placenta.

TESTIS

A sample from a histologically normal testis removed in the treatment of metastatic prostatic adenocarcinoma, was investigated and cells, probably spermatogonia and/or primary spermatocytes, showed ERV3 positivity, while the remainder including Sertoli and Leydig cells, were negative. Thus ERV3 expression seems to be restricted to specific stages of differentiation.

Both the investigated seminomas were either negative or had negligible ERV3 expression.

HUMAN EMBRYOS - 12 WEEKS OF GESTATION

Hybridization was performed on sagittal sections of a human embryo. Low levels of ERV expression were detected in most

organs. However high expression was found in the following locations: Adrenal glands, Rathkes pouch and (probably) the pituitary gland, nerve roots, primitive glomeruli, peripherial ganglia and structures of the primitive skin. Nevertheless, this is a preliminary investigation and will be followed by a detailed study involving several fetuses.

DISCUSSION

In this communication we have attempted to address the question of whether the observed high expression of ERV3 env in placenta specifically correlates with pregnancy, indicating that the retroviral genome can only be expressed in syncytiotrophoblasts which are the result of fertilization. Our ISH data demonstrate, however, that it is probably the fusion process itself and not the fertilization which correlates with ERV3 env expression. Our previous reports concerning the lack of ERV3 env expression in the hydatidiform moles and choriocarcinomas are perhaps due to scarcity of such fused cells in these tissues. The cc cell lines were predomonantly composed of cytotrophoblasts but a few syncytiotrophoblasts were also seen.

Our conclusions concerning ERV3 env expression can be further substantiated by the fact that syncytiotrophoblasts, both in ovarian and testicular carcinomas, expressed ERV3 env. In these cases it can be excluded that the choriocarcinomas are tumours derived from a fertilized ovum. The correlation between env expression and cell fusion has been shown to exist in the invitro differentiated trophoblasts when isolated trophoblasts from term placentas were allowed to mature in vitro and fuse to form syncytiotrophoblasts (3). In these model systems env expression increases in proportion to the degree of cell fusion.

It is still unclear whether env expression is a consequence of cell fusion or is causatively involved in the fusion process. However, these questions are being addressed at our laboratory by inhibiting the ERV3 env expression by means of antisense techniques in combination with antibodies raised against structural viral proteins. The effects on the fusiogenic property of cytotrophoblasts during in vitro differentiation will be studied.

The ERV3 env transcripts contain sequences that can encode not only the transmembrane protein p15E and the envelope gp70 but also nonviral sequences which were found to code for a human zinc finger protein related to the Drosophila transcription factor Kruppel (7). This means that there exists (a) genetic information for proteins with immunosuppressive properties (p15 E) and fusiogenic capacity (gp70) and (b) a Kruppel related transcription factor which may

function as a suppressor "DNA silencer" in the same transcript. This may support the hypothesis that the ERV3 locus (or a related locus) could be involved in normal implantation, immunosuppression and control of trophoblast invasiveness. This is an attractive, yet unproven, hypothesis.

This raises the question of the origin of retroviruses in general and to the env gene in particular: these subjects have been elegantly reviewed several times (2, 15, 16). Since 10% of the human genome consists of sequences that are the result of reverse transcription, retroelements have contributed significantly to the evolution of human DNA. ERVs show striking similarities to invertebrate transposable elements and, according to a recent hypothesis ERV genomes are of two different types. The first evolved from retroelements which through transpositions and recombinations led to the creation of retroviruses by a stepwise process. Since retrotransposons miss an env gene they can only transpose within individual cells.

The addition of an env gene will lead to a retrovirus - retroelement that can move to other cells within the same individual or between species (interspecies transmission) and thus result in the second type of retroelement as a sequelae of an exogenous infection.

According to this hypothesis env genes should also exist as "normal" cellular genes under the control of cellular promotors. This has not been shown but early data from studies in mice reflect that env proteins are expressed to a high degree in reproductive tissues especially in the epididymis, without the production of corresponding numbers of retroviral particles(13). We do not know as yet whether the expression of ERVs in human tissues (especially embryonic and reproductive) indicates that they have a function in differentiation or whether they are expressed as a consequence of differentiation. In the latter case, they could be regarded as inert remnants of germ line infections that occurred during evolution and survived in the genome as parasites since they were difficult to eliminate but gave no selective advantage to the host animal (2).

Research with human ERVs has been hampered by a lack of reagents reactive with human ERVs in general and envelope proteins in particular because of constant failure to purify and characterize a human envelope protein. However, we have shown indirectly that the ERV3 env gene is translated to proteins. These data were obtained by immunohistochemical investigations using antisera directed against synthetic peptides and fusion proteins, which were based on the sequence of the env (10).

In order to study the role of ERV envs in cell fusion and immunosuppression, it will be necessary to block the expression of specific ERVs by antibodies, synthetic peptides and/or disrupt selected env genes in suitable cell lines. We think that ERV3 is a

good candidate for such experiments since it is a single copy virus and we have found that it is differentially regulated during induced differentiation of the human monocytic cell line U937 (1). However, there are also several other ERVs expressed in the human reproductive tissue including some of the multicopy genomes (reviewed in12 and 17). Even if ERV3 env does not have an effect related envs could be of importance.

ACKNOWLEDGEMENTS

These studies were financially supported by grants from the Swedish Cancer Society, project number 2037-B93-10 XCC

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