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ERV3 Human Endogenous Provirus mRNAs Are Expressed in Normal and Malignant Tissues and Cells, But Not in Choriocarcinoma Tumor Cells

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Messenger RNA expression of a human endogenous provirus, ERV3, has been characterized in 170 specimens of normal and malignant human tissues and cells. In contrast to the high expression in first-trimester and full-term placental chorionic villi, most other human tissues expressed ERV3 mRNAs at a level of 2–30% of placenta. However, ERV3 mRNAs were not detected in choriocarcinoma tumor cell lines. These studies suggest that the ERV3 provirus may have been preempted for a biological function and disruption of its mRNA expression results in choriocarcinoma.

Key words: human endogenous provirus, choriocarcinoma, proviral mRNA expression

Human DNA contains many integrated copies of retrovirus genomes that are related to the more widely characterized murine- and primate-type C retroviruses. Although a few of these human endogenous proviruses have been molecularly cloned and analyzed at the nucleotide sequence level [1], very little is known about their evolutionary origin or biological function(s). Recently, investigators described the RNA expression of a human type C-related provirus family that is present in human DNA in 70–100 copies per haploid genome [2].

We previously characterized another human endogenous provirus, ERV3, which was isolated from a human recombinant library by hybridization with the *pol* gene of an endogenous retroviral clone from chimpanzee and the baboon endogenous retrovirus long terminal repeat (LTR) [3]. ERV3 is a full-length provirus that contains a nondefective *env* glycoprotein gene [4] and functional promoters in the LTRs [5]

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(unpublished studies). In addition, the LTRs contain two glucocorticoid regulatory element sequences upstream from the promoter [5]. Because only a single copy of ERV3 is present in human DNA, ERV3 is useful for studying the expression and potential function of a specific human provirus. ERV3 was localized to human chromosome 7 [3].

To determine whether the ERV3 provirus is expressed in humans, we isolated RNA from various tissues of first-trimester abortuses and analyzed the RNAs by Northern blot hybridization [6]. We found that three major ERV3 mRNAs of 9, 7.3, and 3.5 kilobases (kb) were expressed in first-trimester placental chorionic villi but that only the 9-kb and low amounts of the 3.5-kb mRNAs were expressed in the embryo. RNA of full-term placental chorionic villi revealed a pattern identical with that of first-trimester placenta. We showed by Northern blot and S1 nuclease mapping analyses that the three ERV3 mRNAs were all spliced, *env*-containing RNAs [6]. The 9- and 7.3-kb mRNAs extended through the ERV3 3′ LTR to a second splice donor site approximately 370 nucleotides (nt) downstream from the 3′ LTR and contained, respectively, 5.5 or 3.8 kb of human genomic sequences [6]. The nature of these human sequences is presently under investigation. The 3.5-kb ERV3 mRNA was characterized by Northern blot analysis, S1 mapping, and sequence analysis of a cDNA clone isolated from a human 20-week-old fetal liver library [4,6]. The 3.5-kb mRNA was a typical subgenomic, spliced proviral message containing *env* but not *gag* or *pol* sequences and terminating in the 3′ LTR.

We have undertaken an intensive investigation of ERV3 mRNA expression in human specimens to determine whether and to what extent normal expression is altered in malignant tissues and cells. In this study, we noted a dramatic absence of ERV3 mRNA expression in choriocarcinoma tumor cells.

MATERIALS AND METHODS

Tissue and Cells

Full-term human placentas were obtained from Frederick Memorial Hospital (Frederick, MD). Other human tissues were obtained from the Department of Pathology, University of Uppsala (Uppsala, Sweden). Human cell lines were obtained from Sloan-Kettering Institute for Cancer Research (Rye, NY). Choriocarcinoma cell lines ElFa and DoSmi were the kind gift of Dr. Roland Pattillo, Medical College of Wisconsin, (Milwaukee, WI). The MCF-7 cell line was kindly furnished by Dr. Sam C. Brooks, Michigan Cancer Foundation (Detroit, MI). The U-937 cell line was the kind gift of Dr. Kenneth Nilsson, University of Uppsala (Uppsala, Sweden). The choriocarcinoma cell line, GCC-SV, was kindly provided by Dr. Wolfgang Rettig, Memorial Sloan-Kettering Cancer Center (New York, NY).

Preparation of Nucleic Acids

Total RNA from tissues was prepared by the lithium chloride-urea method as previously described [6] with the modification that the first RNA pellet was washed with 3 M lithium chloride to remove contaminating DNA. Total RNA from tissue culture cells was also prepared by the lithium chloride-urea method, as above, except that cells were homogenized by vortexing. DNA was isolated from the supernatant fluid of the same preparation after the first RNA centrifugation. DNA was precipitated with ethanol, dissolved in TE (10 mM Tris, pH 7.5, 1 mM EDTA), treated with

phenol-$CHCl_3$, and reprecipitated with ethanol. High-molecular-weight DNA from tissues was prepared in the standard manner [7].

Northern and Southern Blot Hybridization

Northern blot hybridization was as previously described [6,8]. For Southern analysis, 5 μg of DNA was digested with a restriction enzyme, and fragments were separated by electrophoresis in an agarose gel and transferred to nitrocellulose [9]. Hybridization conditions were the same as that for Northern blot hybridization [6]. After hybridization, the filters were washed twice with 2 × SSC, 0.1% SDS at room temperature for 10 min, in 1 × SSC, 0.1% SDS at 68°C for 1 hr, and finally in 0.1 × SSC, 0.1% SDS at 68°C for 1 hr. The filters were dried and autoradiographed with Kodak XAR-5 film with intensifying screens for 2 days.

RESULTS

To determine whether transcription of the human endogenous provirus, ERV3, is tissue specific and to compare the transcriptional levels of ERV3 in normal and malignant tissues, we surveyed ERV3 expression in 158 specimens of 31 different tissues, including 52 specimens from malignant tissues. Total RNA was isolated from each tissue and initially tested for ERV3 mRNAs by dot blot hybridization with a plasmid probe containing the entire ERV3 *env* glycoprotein and a portion of the transmembrane protein-coding sequence (Fig. 1). The highest level of expression was observed in the placental chorionic villi [6]. ERV3 transcripts represented 0.03–0.05% of the total mRNA in first-trimester and full-term placenta [6]. The composition of these mRNAs is summarized in Figure 1.

The level of ERV3 mRNA expression in most other human tissues was 10% or less than that of chorionic villi. However, several specimens revealed higher levels of expression including normal thymus, breast, testis, and gallbladder tissues and malignant breast, glioma, pancreas, stomach polyp, lung, rhabdomyosarcoma, and Wilms' tumor tissues. ERV3 expression in these specimens was 10–30% of that in placental chorionic villi (Table I). RNA from some of these tissues was analyzed by Northern blot hybridization using the ERV3 *env* probe as described above (Fig. 2). Each lane of the formaldehyde-agarose gel was loaded with 10 μg of total cellular

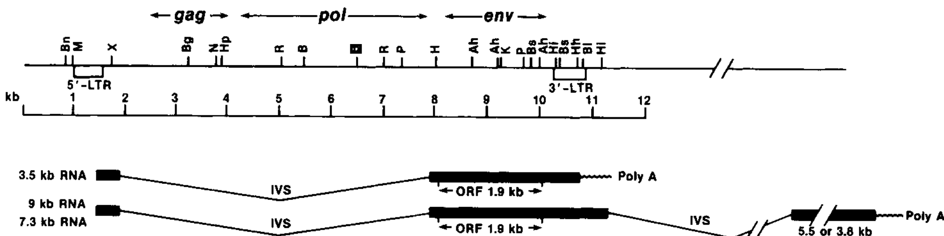

Fig. 1. Structure of the three ERV3 mRNAs. **Top:** Genetic and partial restriction map of the ERV3 provirus. **Bottom:** Exons are represented by thick bars; intervening sequences (IVS) by lines. Abbreviations: ORF, open reading frame; Ah, *Aha*III; Av, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; Bl, *Bal*I; Bn, *Ban*I, Bs, *Bst*NI; H, *Hind*III; Hh, *Hha*I; Hi, *Hin*fI; Hp, *Hpa*I; K, *Kpn*I; M, *Mn*lI; N, *Nru*I; P, *Pst*I; R, *Eco*RI; X, *Xma*I.

TABLE I. ERV3 mRNA Expression in Human Tissues and Cells

Normal tissue	
Placental chorion	40 first trimester, 6 term
High-expression group (10–20% of placental chorion)	4 gallbladder, 1 thymus, 1 testis, 1 breast
Low-expression group (2–10% of placental chorion)	11 endometrium, 10 stomach, 3 liver, 3 small intestine, 2 ovary, 2 myometrium, 2 epididymis, 2 prostate, 2 thyroid gland, 2 colon, 1 spleen, 1 lymph node, 1 skin, 1 pancreas, 1 bladder, 1 duodenum, 1 parotid, 1 testis
Malignant tissue	
High-expression group (10–30% of placental chorion)	8 breast, 2 glioma, 2 pancreas, 1 stomach polyp, 1 lung (small cell), 1 rhabdomyosarcoma, 1 Wilms' tumor
Low-expression group (2–10% of placental chorion)	11 lung, 6 lymphoma, 4 stomach, 2 rectum, 2 kidney, 2 colon, 1 liver, 1 prostate, 1 gallbladder, 1 parotid, 1 bile duct, 1 duodenum, 1 bladder, 1 invasive hydatidiform mole
Cultured cells	
High-expression group (10–30% of placental chorion)	Monocytic leukemia, U937; 2 endometrial carcinoma, 2 cervical carcinoma, 1 breast carcinoma, MCF-7
Low-expression group (<2% of placental chorion)	6 choriocarcinoma

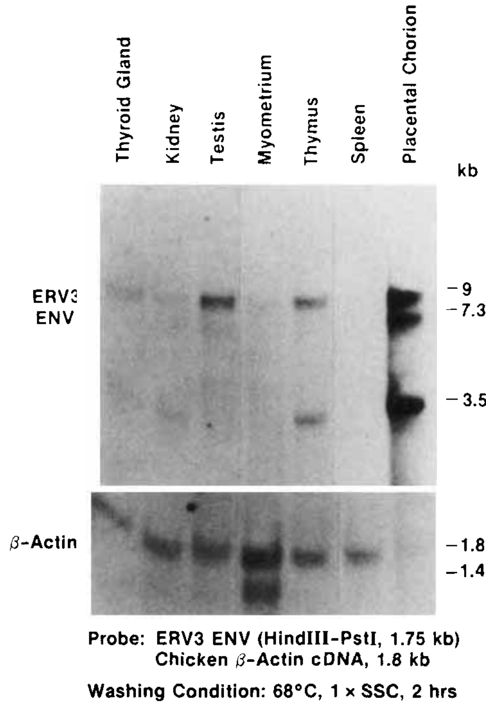

Fig. 2. Northern analysis of ERV3 mRNAs in human tissues. **Top**: Hybridization with ERV3 isolated 1.75-kb *env*-containing fragment, *Hin*dIII-*Pst*I; map position 8-9.7 (see Fig. 1). **Bottom insert:** Rehybridization of filter with chicken β-actin plasmid probe.

RNA. As a control, gels were stained with ethidium bromide before transfer to nitrocellulose to visualize the ribosomal RNAs. This provided a reliable means of estimating the relative quantity and quality of each RNA. In addition, filters were rehybridized with a chicken β-actin probe without removal of the ERV3 probe. The presence of the 1.8-kb β-actin mRNA and in some tissues the 1.4-kb β-actin mRNA, provided an independent assessment of RNA quality, although some differences in expression were noted among various tissues (Fig. 2). RNA from normal testis, thymus, and breast tissues and malignant breast, glioma, rhabdomyosarcoma, and Wilm's tumor tissues contained high levels of ERV3 mRNAs (Figs. 2, 3). In contrast, RNA from normal thyroid gland, kidney, myometrium, and spleen tissues and several malignant tissues, including leiomyosarcoma and sarcoma, contained low levels of ERV3 mRNAs. A striking feature of ERV3 expression in all these tissues was the absence of the 7.3-kb mRNA that was observed in the placental chorionic villi (Figs. 2, 3). Expression of the 7.3-kb mRNA is apparently specific for the placental chorion.

A high level of ERV3 mRNAs was also noted in some of the 12 human cell lines tested including the monocytic leukemia line, U937; breast cancer, MCF-7; two endometrium carcinomas, HEC-1B and An3CA; and two carcinomas of the cervix, C-33A and SiHa (Table 1). When analyzed by Northern blot hybridization, HEC-1B, AN3CA, and C-33A cells expressed the 9- and 3.5-kb ERV3 mRNAs, whereas SiHa cells expressed only the 3.5-kb mRNA (Fig. 4). Like nonplacental tissues, these human cell lines did not express the 7.3-kb mRNA. In contrast to endometrial and cervical carcinoma cells, two choriocarcinoma cell lines, BeWo and JAR, expressed almost no ERV3 mRNAs (Fig. 4). It is clear from the ethidium bromide-stained

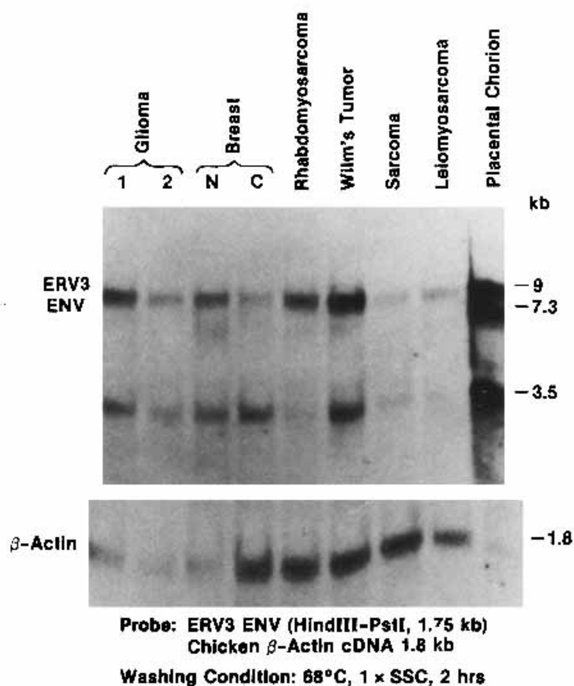

Fig. 3. Northern analysis of ERV3 mRNAs in human tissues. **Top:** hybridization with ERV3 1.75-kb *env* fragment. **Bottom insert:** Rehybridization of filter with chicken β-actin plasmid probe.

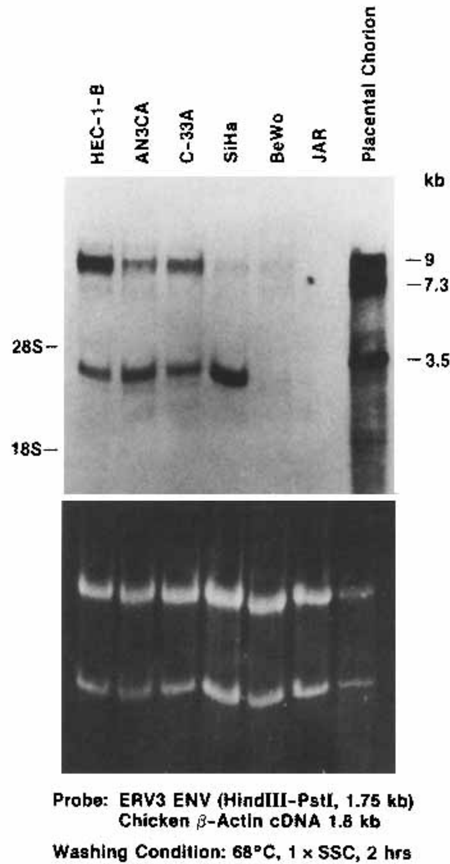

Fig. 4. Northern analysis of ERV3 mRNAs in human cells. **Top:** Hybridization with ERV3 1.75-kb *env* fragment. **Bottom:** Ethidium bromide staining of ribosomal RNAs in gel before transfer of RNA to nitrocellulose filter.

fluorescence pattern of the ribosomal RNAs (Fig. 4, bottom) that the absence of ERV3 mRNAs was not the result of differences in the amounts of RNA present in those lanes, nor to RNA degradation. To determine whether the absence of ERV3 mRNAs is a general feature of choriocarcinomas, we examined the RNA of four other choriocarcinoma cell lines, ElFa, DoSmi, GCC-SV, and JEG-3. Like JAR and BeWo, these choriocarcinoma lines also expressed almost no ERV3 mRNAs as determined by Northern and dot blot analysis, although long autoradiographic exposure revealed very low levels of ERV3 mRNAs in the ElFa line [6]. We were unable to obtain choriocarcinoma tumors; however, we obtained an invasive hydatidiform mole tissue. Invasive moles, moles that penetrate deep into the myometrium beyond their usual decidual implantation site, frequently occur following a molar pregnancy. Analysis of RNA from the invasive hydatidiform mole revealed a similar absence of ERV3 mRNA expression [6].

DISCUSSION

Three mRNAs of the human endogenous provirus, ERV3, are highly expressed in the human chorionic villi throughout development [6]. The abrogation of this

expression in choriocarcinoma cells in culture and in an invasive hydatidiform mole tissue may represent the primary etiologic defect in these tumors. In other studies, we found that the absence of ERV3 mRNA expression is not the result of homozygous deletion of the proviral locus as shown by the finding that the ERV3 genome is present and unrearranged in the DNA of 5 choriocarcinoma cell DNAs [6].

Human gestational choriocarcinoma is a malignancy containing trophoblastic cells but no placental villi. The risk of choriocarcinoma is significantly elevated in women with a previous molar pregnancy: In several studies, 29–83% of choriocarcinomas were preceded by complete hydatidiform moles [10]. Complete hydatidiform moles usually result from elimination of the maternal nuclear component and duplication of the paternal chromosomes such that complete moles are homozygous with a 46,XX androgenous karyotype. Occasional dispermic fertilization of an empty egg results in 46,XY or 46,XX genetically heterozygous moles. However, all the choriocarcinomas that have been analyzed are heterozygous, suggesting that choriocarcinoma either does not directly descend from a complete mole, or it results only from the rare heterozygous moles.

Two classes of cellular genes have been associated with various cancers. One class, termed proto-oncogenes, is dominantly associated with malignancies. These elements can cause cellular transformation by disrupting normal growth processes and often result from chromosomal rearrangements or mutations that allow their aberrant expression. A second class of cellular genes may represent recessive suppressors of specific malignancies. These genes were discovered because their chromosomal loci are sometimes deleted in the resulting tumor. The prototype of this gene is a locus in chromosome region 11p13. Homozygous deletion or mutation of this locus can result in any of three embryonal tumors: Wilm's tumor, hepatoblastoma, or rhabdomyosarcoma [11–13]. The presence of immature cells in these childhood tumors suggested that the locus is required for normal differentiation [12]. Another member of this class of genes is the human retinoblastoma-susceptibility gene locus (RB) located in chromosome region 13q14. The gene is abnormally transcribed in retinoblastoma tumors [14, 15]. Because retinoblastoma is primarily a childhood cancer and has characteristics of an embryonal cancer, the RB locus may play a role in normal development [15]. Other recessive loci that may control specific malignancies include bladder cell carcinoma [16], uveal melanoma [17], and bilateral acoustic neurofibromatosis [18].

Although little is known about the molecular basis of choriocarcinoma, this malignancy may not derive from proto-oncogene activation resulting from a specific chromosomal rearrangement. Although several proto-oncogenes were found to be expressed in choriocarcinoma and hydatidiform mole cells, including c-*myc* and c-*ras* [19], and c-*fos* in the BeWo choriocarcinoma cell line [20], normal first-trimester trophoblast cells also express c-*myc* [21] and c-*sis* [22] mRNAs. Furthermore, no consistent chromosomal abnormalities were detected in four choriocarcinoma cell lines [23] or in three choriocarcinoma tumors [24].

The high level of ERV3 mRNA expression in normal placental chorionic villi throughout gestation, and the absence of ERV3 expression in choriocarcinoma cells suggests a possible biological function for an ERV3 gene product. Similar to the functions postulated for the Wilm's tumor and retinoblastoma loci, a possible function of the ERV3 provirus may be to act as a suppressor of tumorigenicity in the placenta. Loss of ERV3 mRNA expression would thus result in choriocarcinoma. Another

possibility is that abrogation of ERV3 mRNA expression and choriocarcinoma susceptibility are pleiotropic effects of another defect, perhaps in a transcription factor. A third possibility is that loss of ERV3 expression may be an effect rather than a cause of choriocarcinoma. These possibilities are currently under investigation.

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