Journal of Virology

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J. Virol. **2006, 80(9):4415. DOI: 10.1128/JVI.80.9.4415-4421.2006.**

JOURNAL OF VIROLOGY, May 2006, p. 4415–4421 Vol. 80, No. 9
0022-538X/06/$08.00+0 doi:10.1128/JVI.80.9.4415–4421.2006

Expression Profiles of Endogenous Retroviruses in Old World Monkeys†

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Received 17 August 2005/Accepted 6 February 2006

Human endogenous retroviruses (HERVs) are a major component of the human genome and an active part of the transcriptome. Some HERVs play vital biological roles, while others potentially contribute to diseases. Many HERVs are relatively new in the primate genome, having entered or expanded after the lineages leading to the platyrrhines (New World monkeys) and catarrhines (Old World monkeys and apes) separated. Most HERVs are active in at least some tissues, though tissue specificity is common for most elements. We analyzed multiple tissues from several Old World monkeys using retroviral *pol*-based DNA microarrays and quantitative PCR methods to determine their ERV expression profiles. The results demonstrate that while many ERVs are active in nonhuman primates, overall the tissue expression specificity is unique to each species. Most striking is that while the majority of HERVs analyzed in this study are expressed in human brain, almost none are expressed in Old World monkey brains or are only weakly expressed.

Human endogenous retroviruses (HERVs) comprise at least 8 to 9% of the human genome. They are associated with both critical biological function such as placental formation (6, 28) and with diseases such as multiple sclerosis (1) and germ cell tumors (15). HERVs can be subdivided into three different classes based on features held in common with specific exogenous retrovirus families, such as foamy viruses, betaretroviruses, and gammaretroviruses. HERV classes can be further subdivided to define individual subfamilies, a useful feature for distinguishing different groups of HERVs from one another. HERVs demonstrate variability at many taxonomic levels. For example, recently, a DNA microarray-based approach was used to elucidate the distribution of HERVs in nonhuman primates (16). While New World monkeys (NWMs) generally lack or have a reduced copy number of most ERV classes, Old World monkeys (OWMs) display a similar, though more restricted, ERV content compared to that of humans. Eight families of gammaretroviruses, nine families of betaretroviruses, and five subgroups of HERV-L elements could be detected in OWMs with a *pol*-specific microarray (16). The differences in HERV distributions between humans and other hominoid apes have been explored in particular detail (for reviews, see reference 35). Members of the HERV-K family have been identified as potentially transpositionally active and polymorphic in human populations (2, 8, 36). Thus, at the DNA level, HERV evolution involves both vertical transmission and de novo integration of elements by retrotransposition or reinfection, whether looking within or among species (3, 4, 26). However, the majority of elements integrated before the OWM and great ape lineages diverged and thus have a similar genomic distribution pattern among catarrhines (16, 10, 27).

HERV variation among species at the transcriptome level is less well characterized. However, in the cases where it has been examined, repetitive DNA and HERVs in particular have been shown to be a component of the human transcriptome (13, 24, 32, 34). While tissue-specific patterns of HERV expression have been demonstrated, all tissues analyzed to date express at least some elements. The most comprehensively studied tissues are those of neurological origin. For example, analysis of 215 human brain RNA samples has demonstrated the brain-specific HERV expression profile (14). A characteristic brain-specific HERV activity profile was observed consisting of the class I family members HERV-E, HERV-F, and ERV9 as well as class II family members HML-2, HML-4, HML-6, HML-9, and HML-10. In addition to these constitutively expressed HERVs, a number of elements demonstrated among-individual variation in expression.

The expression profiles of HERVs are relevant, because a key finding from the several mammalian genome projects undertaken is that among closely related primate species, the genetic differences are not pronounced. For example, common chimpanzees (*Pan troglodytes*) and humans show 95 to 98.5% sequence similarity overall, depending on whether or not insertions and deletions are counted (7). Recent experiments suggest that the fundamental biological differences between primate species are due to differences in gene expression and regulation rather than raw sequence variation. For example, several DNA microarray-based studies have demonstrated that humans show higher expression levels of multiple genes in the brain at the RNA level relative to nonhuman primates (9, 11, 18, 22, 37). This may also hold true at the protein level (11) and may be accounted for in part by methylation differences (12). Tissues other than brain do not show such pronounced overall expression differences among the primates

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† Supplemental material for this article may be found at http://jvi.asm.org.

tested. One hypothesis is that increases in the aerobic requirements of the brain or the increase in cognition in humans relative to other primates are linked to the accelerated gene expression in the human brain (29, 37).

The purpose of this investigation is to determine if HERVs display an expression profile similar to that of nonrepetitive elements in nonhuman primates. Using *pol* gene-based microarrays (32, 33) and quantitative PCR methods (16, 33), we examined the expression of multiple subfamilies of ERVs previously shown to be present in the genomes of OWMs (16) for the OWM species *Mandrillus sphinx* and *Macaca sylvanus* in multiple tissues. For elements that could be detected in both OWM species, the results were compared to the expression profiles determined for humans (14, 33). For most tissues, many ERVs analyzed demonstrated a species-specific tissue expression profile. Of particular note, both the number of active ERV subfamilies and their expression levels were greatly increased in human brain compared to OWM brain samples. Thus, HERVs, like other portions of the human transcriptome, demonstrate a relative increase in brain expression compared to nonhuman primates.

MATERIALS AND METHODS

Primate tissues. Tissue samples from three unrelated (two female and one male) barbary macaques (*Macaca sylvanus*) and a male mandrill (*Mandrillus sphinx*) were obtained from the Gene Bank of Primates, Göttingen, Germany. Sample numbers for each animal were the following: female macaques, 1020 and 1021; male macaque, 1022; and male mandrill, 1023. Brain cortex, skeletal muscle, kidney, liver, heart, and lung were provided for macaque 1020 and the mandrill. Brain, skeletal muscle, and kidney were provided for female 1021 and male macaque 1022 as well. Human kidney and skeletal muscle RNA samples, both of female origin, were obtained from Stratagene (La Jolla, CA). Prefrontal cortex-derived normal human male brain RNA was donated by the Stanley Medical Research Institute's Brain Collection (Bethesda, MD), courtesy of Michael B. Knable, E. Fuller Torrey, Maree J. Webster, Serge Weis, and Robert H. Yolken.

DNA and RNA preparation and reverse transcription. RNA was isolated using an RNeasy Mini kit (QIAGEN, Hilden, Germany) and made DNA free using an RNase-free DNase set (QIAGEN, Hilden, Germany). One microgram of each sample was reverse transcribed using the Superscript First-Strand Synthesis System for RT-PCR kit (Invitrogen, Karlsruhe, Germany) and random hexamers according to the manufacturer's instructions. Controls without reverse transcriptase (RT) were also done to ensure that all samples were DNA free. DNA was prepared from a female macaque (1020) and the male mandrill sample using a QIAamp DNA Mini kit (QIAGEN, Hilden, Germany).

Multiplex PCR for amplification of cDNAs. In order to prevent amplification of contaminating DNA, all PCRs were carried out in a PCR hood in a room dedicated to RNA extraction and PCR setup. No DNA or PCR products ever enter the room. RNA work and PCR setup were performed after 10 to 15 min of UV irradiation of the work area to destroy any potential contaminating DNA. The PCR primers used in this study are based on conserved regions of the *pol* gene for the different HERV classes and are described in references 32 and 33. Cy3-labeled DNA probes were synthesized by PCR in a total volume of 50 μl containing 2 μl cDNA (100 ng), 5 μl 10× Mg-free buffer (Promega, Madison, WI), 2 mM $MgCl_2$, 0.25 mM of deoxynucleotide triphosphate mixture, 1.25 U of *Taq* polymerase (Promega, Madison, WI), and 3 μl of multiplex primer mixture (32). Amplification was performed in a DNA Thermal Cycler 9600 (Perkin Elmer Cetus, Norwalk, CT), starting with an initial denaturation step with 5 min at 94°C and then 35 cycles of 94°C for 30 s, 50°C for 3 min, and 72°C for 2 min. A final extension step of 10 min at 72°C was included. Genomic DNA (100 ng) from both a macaque and the mandrill was also assessed, and elements not detected at the DNA level were excluded from further analysis. To increase signal intensity, each primate multiplex PCR was performed in duplicate and the PCRs were pooled prior to microarray hybridization. A water negative control or reverse-transcribed tissues omitting the reverse transcriptase (no RT) were included to monitor contamination. Five microliters of each reaction was visualized on an ethidium bromide-stained 2% agarose gel to ensure that amplification had occurred and that the water control was negative. In all cases, primate DNAs and cDNAs generated strong PCR products and the water and no-RT controls were negative.

Design of DNA microarrays and hybridization procedures. The DNA microarray consists of 90-bp RT sequences specific for subfamilies of human class I, II, and III HERVs (20). It should be noted that the microarray is designed to detect subfamilies of elements, and thus a positive signal may represent many unique related elements as opposed to individual elements. The layout of the chip is detailed in reference 32, with additional newly characterized HERV *pol* sequences spotted as described in reference 33. Capture probes scored in this study, including sequences corresponding to five housekeeping genes and the mammalian ERVs baboon endogenous virus (BaEV) and gibbon ape leukemia virus (GaLV), are listed in Table S1 in the supplemental material. Hybridization was performed for each primate sample in duplicate. Water control hybridizations were negative. As each microarray is spotted in triplicate, a total of six spots were scored for each HERV subfamily for each sample. Hybridization procedures are described in detail in reference 16. The only notable differences in procedure is that rather than using hybridization chambers, coverslips were glued to the microarray and the hybridization was carried out under the coverslip to concentrate the samples and maximize the amount of PCR product in contact with the capture probes. After hybridization, coverslips were removed and subsequent handling was performed as described previously (16, 32, 33). DNA from the OWM animals 1020 and 1023 was also hybridized to the microarrays to confirm the previous results (16) for the mandrill and to determine which ERVs are detectable in *M. sylvanus* using the human-based DNA microarray. DNA-positive HERV subfamilies were then scored for the OWM tissue RNAs.

Scanning and microarray evaluation. The program GMS 418 Array Scanner Software, version 1.51.0.42 (Genetic MicroSystems, Inc., MWG the Genomic Company, Ebersberg, Germany), was used to generate TIFF files for evaluating background, contamination, and presence or absence of each spot in a given sample. Contamination was not detected in any experiment. Results were imported into Microsoft PowerPoint for alignment of hybridization results. If a signal was detected by visual inspection for a given HERV in all six repetitions, it was scored as positive and is indicated as such in Fig. 1.

Quantitative real-time PCR. LightCycler Real-Time PCR analysis using FastStart DNA MasterPlus SYBR Green I (Roche Molecular Biochemicals, Mannheim, Germany) was performed on cDNA derived from reverse-transcribed DNA-free RNA samples according to the manufacturer's instructions. All primer pairs were tested using different amounts of target cDNA (1 ng, 10 ng, and 100 ng) to ensure that the amplification efficiencies of the different primer pairs were close to equal (data not shown). ERV-specific primers were designed such that for each ERV, one primer matched the capture probe sequence used in the corresponding microarray experiments. The second primer was located 100 to 200 bp downstream of the first primer. Where information was available from primate sequences, primers were designed to match all possible sequences. HERV-E (E4-1), HERV-K (HML-3), HERV-W, and HERV-L were analyzed. Experiments for each gene were performed in triplicate. A second set of human cDNAs and two independent control genes were used to ensure that results were not individual or control gene dependent. Cycling conditions on a Roche LightCycler instrument were as follows: initial denaturation step at 95°C for 10 min, and then 40 cycles at 95°C for 10 s, 60°C for 5 s, and 72°C for 10s. After 40 cycles, melting curves were generated for the final PCR products by decreasing the temperature to 65°C for 15 s followed by an increase in temperature to 95°C. Fluorescence was measured at 0.2°C increments. Relative quantification of HERV *pol* transcription was performed using LightCycler Software (version 3.5; Roche Molecular Biochemicals, Mannheim, Germany) and housekeeping genes as calibrators for normalization (30). Results were analyzed using Microsoft Excel 2000. Five microliters of each reaction was run on ethidium-stained agarose gels (1.5% 1× Tris-acetate-EDTA; data not shown) to confirm that a specific product was measured. Data was normalized to RPII (RNA polymerase II) and TBP (TATA box binding protein) housekeeping genes (30). For comparing ERV expression among tissues within an individual animal, the ΔC_t representing the threshold point value (C_t) of a given HERV family normalized to the C_t level of the housekeeping gene was used. In order to determine the fold difference in expression among species, data normalized to the control gene RPII or TBP was analyzed using the $2^{-\Delta\Delta Ct}$ method (25) to compare relative human expression levels to that of the OWMs for each HERV tested quantitatively (17, 30). As normalization with RPII and TBP yielded similar results, only transcriptional activity normalized to RPII expression levels is shown. The results do not provide an absolute measure of RNA quantity but a relative fold difference between samples. Primers used were HERV-E E4-1 (forward, 5′ GGTGTCACTACTCAATACAC 3′; reverse, 5′ GCAGCCTA GGTCTCTGG 3′), HML-3 Seq26 (forward, 5′ CTGCAGCCTGCTAAGCG 3′; reverse, 5′ CACTGTGAAAATTTTTTACGAG 3′), HERV-W (forward, 5′ TGAGTCAATTCTCATACCTG 3′; reverse, 5′ AGTTAAGAGTTCTTGG

HERV	Genomic DNA		Skeletal muscle [a]					Heart			Liver		
	M. sylvanus 1020	*M. sphinx*	*M. sylvanus* 1020	1021	1022	*M. sphinx*	Human	*M. sylvanus* 1020	*M. sphinx*	Human	*M. sylvanus* 1020	*M. sphinx*	Human
Class I													
HERV-FRD		●					●			●			●
HERV-E[b]	●	●		●	●	●	●	●	●	●	●	●	●
HERV-W[b]	●	●		●	●	●	●	●	●	●	●	●	●
ERV-9	●	●		●	●	●	●	●	●	●	●	●	●
HERV-F	●	●				●	●		●	●		●	●
Class II													
HML2	●	●											●
HML3[b]	●	●	●	●	●	●	●	●	●	●	●		●
HML4	●	●					●			●	●		●
HML6	●	●			●		●			●			●
HML10	●	●	●	●	●	●		●	●		●	●	
Class III													
HERV-L[b]	●						●			●			
Mammalian													
BaEV	●	●	●	●	●	●		●	●		●	●	
GaLV	●	●	●	●	●	●		●	●		●		

HERV	Kidney [a]					Brain [a]					Lung		
	M. sylvanus 1020	1021	1022	*M. sphinx*	Human	*M. sylvanus* 1020	1021	1022	*M. sphinx*	Human	*M. sylvanus* 1020	*M. sphinx*	Human
Class I													
HERV-FRD					●					●			●
HERV-E[b]	●	●	●	●	●			●		●	●	●	●
HERV-W	●	●	●	●	●					●	●	●	●
ERV-9	●	●	●	●	●			●		●			●
HERV-F					●					●			●
Class II													
HML2					●					●			●
HML3[b]	●	●	●		●		●			●	●	●	●
HML4	●		●		●					●			●
HML6					●					●			●
HML10	●	●	●	●		●	●	●	●	●	●	●	
Class III													
HERV-L[b]													
Mammalian													
BaEV	●	●	●	●		●	●	●	●		●	●	
GaLV	●	●	●	●							●	●	

FIG. 1. Summary of the expression profiles for ERV families examined in each sample. Tissues and HERVs examined, including the mammalian ERVs BaEV and GaLV, are indicated. The results are shown for the three macaques and the one mandrill examined in this study; results for genomic DNA are also shown and are shaded to distinguish them from the RNA profiles. Human profiles are taken from reference 33. Tissue samples and HERVs analyzed by quantitative PCR are indicated by superscript "a" and "b", respectively. ERV taxa were classified as active if at least one member of the taxon was positive.

GTGG 3′), HERV-L (forward, 5′ CTTCAGCTGGCAAGGCC 3′; reverse, 5′ CC AGTGTGATATCTTGTGGC 3′), ERV-L (forward, 5′ TGTCCATAAGGCCCA CCAGA 3′; reverse, 5′ TGGACCAGTGTGATATCTTG 3′), TBP (forward, 5′ TTCGGAGAGTTCTGGGATTGTA 3′; reverse, 5′ TGGACTGTTCTTCACTCT TGGC 3′), and RPII (forward, 5′ GCACCACGTCCAATGACAT 3′; reverse, 5′ GTGCGGCTGCTTCCATAA 3′).

RESULTS

Analysis of ERV activity in primate tissues using retrovirus-specific DNA microarrays. A nonquantitative human *pol*-based DNA microarray system was used to analyze multiple tissues

for several HERV subfamilies from two OWM species. Fifty representatives of 20 major HERV lineages are represented on the microarray (14). Based on both previous experiments with the same species in the case of the mandrill and related species in the case of the macaques at the DNA level (16), the analysis of all HERVs was not possible, as some HERV subfamilies are apparently absent or at very low copy numbers in most OWMs compared to humans (16). Only ERVs that were tested in human tissues previously (33) and that were positive at the DNA level in OWMs in previous experiments (16) and in the experiments reported here were scored for their presence in the nonhuman primate transcriptomes. Thus, HERV-FRD, HERV-E, HERV-W, ERV9, HERV-F, HML-2, HML-3, HML-4, HML-6, HML-7, HML-9, HML-10, and HERV-L were examined, whereas HERV-I, HERV-T, HERV-H, HML-1, HML-5, and HML-8 were not. *M. sylvanus* in this study differed from *M. mulatta* (16) in that HERV-FRD was positive for *M. mulatta* and HML-4 and HML-6 were negative. The results for *M. sphinx* in this study were similar to those in reference 16, with the exception of HML-4. It should be noted that a positive signal for a given HERV (Fig. S1 and S2 in the supplemental material) does not represent single elements, as the microarray is designed to detect entire subfamilies which may represent many closely related elements.

Class I or gammaretrovirus-like ERVs were detected in all represented human tissues (Fig. 1). HERV-E, HERV-W, and ERV-9 were detected in macaque and mandrill skeletal muscle, heart, liver, and kidney (Fig. 1 and Fig. S1). One female macaque (1020), however, was negative for these elements in skeletal muscle. In lung, ERV-9 was not detected in either OWM. Brain was negative for both OWM species for all three elements except for macaque 1022, which gave a faint positive signal in brain tissue for HERV-E and ERV-9. Of the remaining class I elements tested, HERV-FRD was not detected in either OWM, and HERV-F was detected in mandrill skeletal muscle, heart, and liver. Neither element was detected in brain.

Class II or betaretrovirus-like ERV elements HML-3, HML-4, and HML-6 were expressed in all human tissues studied. HML-2 was not detected in human muscle or heart, and HML-10 was only observed in human brain. For the macaques, HML-3 and HML-10 transcripts were detected in all represented tissues, though not in all macaques tested. The two elements were similarly distributed in the mandrill with the exception of HML-3, which was not detected in liver, kidney, or brain (Fig. 1 and Fig. S2). HML-10 was the only ERV active in all nonhuman primate brain samples tested. HML-3 was only weakly active in macaque 1021 in the brain but was otherwise negative for OWMs. HML-4 was only detected in macaque liver and kidney. HML-2 was not expressed in any OWM tissue. HML-6 was only observed in one macaque skeletal muscle sample (1022). No class III foamy virus-like HERVs were detected in any OWM tissue, although they are active in human skeletal muscle and heart. From the microarray data, the most ERV expression-poor tissue tested in OWMs was brain (Fig. 1).

In addition to human endogenous retroviruses, mammalian ERVs, human exogenous retroviruses, i.e., human immunodeficiency virus, and other nonhuman viruses, such as murine leukemia virus, were included in the microarray analysis. Only GaLV and BaEV transcripts were detected. BaEV (baboon endogenous virus) was expressed in all macaque and mandrill tissues tested, including brain. This element class is restricted to OWMs and was not detected in human DNA or RNA (16, 33). Similarly, GaLV (gibbon ape leukemia virus) was expressed in all tissues of macaque except brain. The same was true for the mandrill tested, except that in addition to brain, liver did not express the element.

Quantitative PCR analysis of ERV transcription. PCR primers for LightCycler were designed based on the capture probe sequences of the *pol* gene-based DNA microarray and conserved regions 100 to 200 bp downstream. Thus, the results are both methodologically and PCR primer independent of the DNA microarray results. Although LightCycler-based methods are generally more sensitive than microarrays, they detect a more limited subset of HERVs due to the higher specificity of the primers used (13, 33). Thus, the results of the independent methods should demonstrate similar trends but may not yield identical results. Primers were tested on macaque and mandrill DNA to ensure that they could detect the desired elements before attempting tissue RNA analysis. Two forms of analysis were performed depending on the comparison desired. For among-tissue comparisons within individuals of each species, the relative ratio of ERV to control gene RPII (ΔC_t) was determined, and the lowest ratio among the tissues, representing the highest expression, was set arbitrarily to a value of 1 for each tissue tested. All ratios were then normalized with respect to the lowest ratio (an analysis similar but not identical to that in reference 23) (Fig. 2A, B, and C). To compare the relative fold HERV expression differences between different species, the $2^{-\Delta\Delta Ct}$ method (17, 25) was employed, whereby the OWM control gene (RPII) normalized ratios were set as the baseline expression level and compared to the human RPII control gene normalized values, yielding the fold change in expression difference (Fig. 2D, E, and F). A second set of human cDNAs and a second control gene (TBP) were also tested, and the results remained consistent irrespective of the control gene chosen or the human individual used for relative comparison (data not shown).

Confirming the microarray results, class III HERV-L sequences were not detected using primers designed to detect human HERV-L (33). However, mandrill DNA also failed to amplify with these primers. Therefore, a second set of primers was designed based on the conservation between NWM ERV-L elements in the database (5) (GenBank accession numbers AJ233633 to AJ233643 and AJ233674) and human HERV-L elements (GenBank accession numbers AJ233628 to AJ233632 and AJ233673). The primers yielded strong PCR products from mandrill and macaque DNA. However, no reverse transcription-PCR product was detected in any of the six tissues tested from either animal, nor was ERV-L detected in any of the tissues of the additional two macaques (1021 and 1022) tested (data not shown).

HERV-W, HERV-E, and HERV-K (HML-3) were each examined for their expression levels in skeletal muscle, kidney, and brain for the mandrill sample, the three macaque samples, and human samples. Figure 2A, B, and C illustrates that the tissue specificity of HERV expression patterns were most similar between macaques and humans, although the relative expression levels (macaque versus human; Fig. 2D, E, and F) differ substantially. Consistent with the microarray results, HERV-E, which was not detected for macaque 1020 in muscle

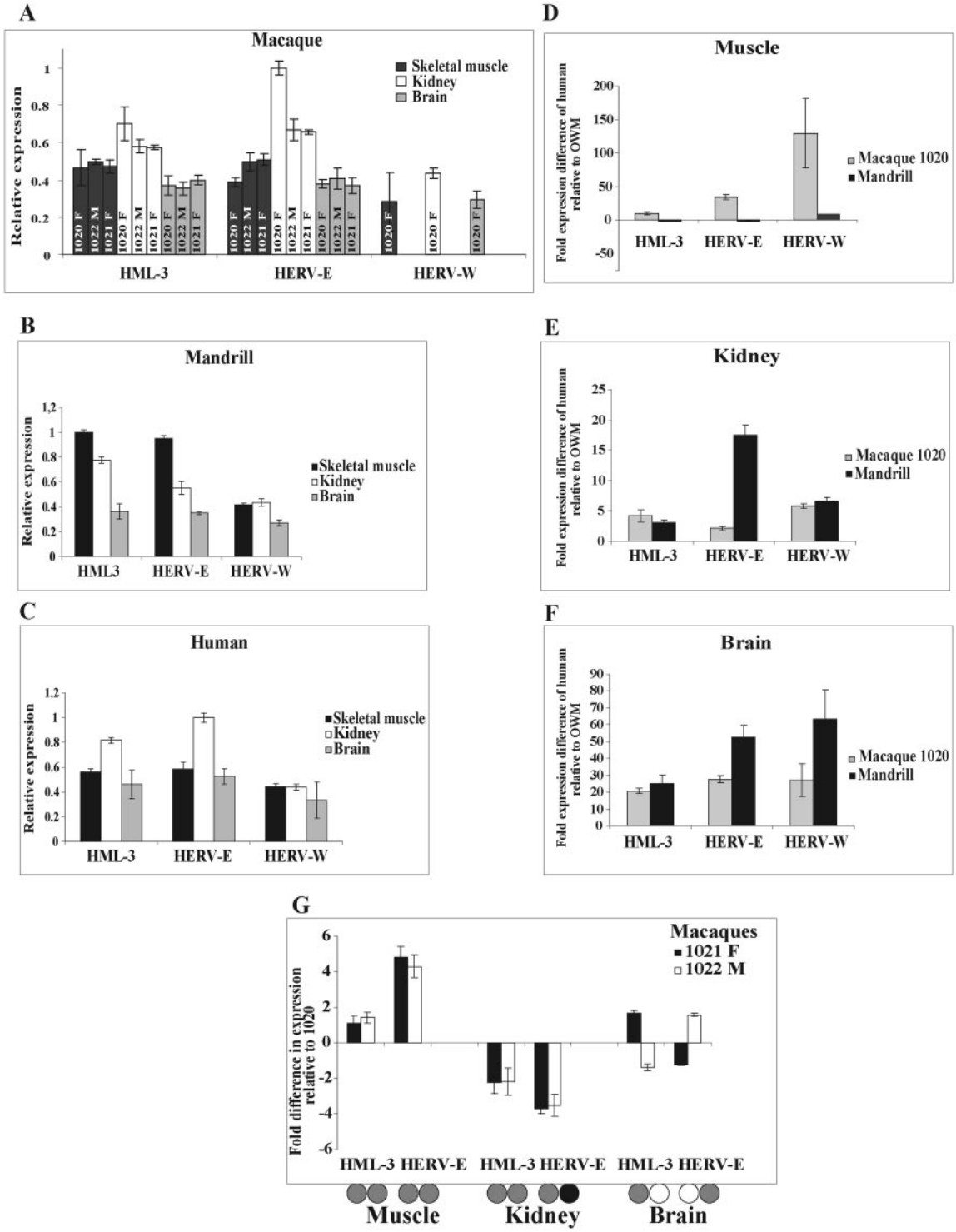

FIG. 2. Real-time PCR quantification of ERVs in human, mandrill, and macaque tissues. The threshold value (C_t) data of HERV-K (HML-3), HERV-E, and HERV-W were normalized to the RPII house keeping gene (ΔC_t) for each tissue in (A) macaques 1020, 1021, and 1022, (B) mandrill 1023, and (C) humans. Male and female macaques are designated by an "m" for male or "f" for female after the respective sample number. The lowest ΔC_t value representing the strongest expression of the corresponding element per tissue was arbitrarily set to a value of 1, and all ratios were normalized to give a value between 0 and 1. The standard error is shown. To compare relative fold expression differences among species, the normalized relative expression values of the OWM ERVs HERV-K (HML-3), HERV-E, and HERV-W were compared to that of human tissue ($2^{-\Delta\Delta Ct}$ method [25], also using RPII to normalize the data). Using either macaque 1020 or mandrill as the baseline, each ERV for each tissue in a mandrill and macaque sample was evaluated for either higher, lower, or the same relative expression level as that seen in human tissue. The *y* axis shows the fold difference expression level of human tissue relative to that of each primate. Standard error bars are shown. Skeletal muscle, kidney, and brain are shown in panels D, E, and F, respectively. (G) The fold difference expression (the *y* axis) calculated using the $2^{-\Delta\Delta Ct}$ method for HML-3 and HERV-E among macaques 1020, 1021, and 1022 are shown, whereas macaque 1020 was used as the expression baseline for comparison. The microarray results are summarized below each column with white, gray, and black circles representing absence of signal, weak signal, and strong signal, respectively.

on the microarray, demonstrated a lower HERV:control gene ratio than did macaque 1021 or 1022. Mandrill differed from macaques and humans in showing a higher relative expression of HML-3 and HERV-E in skeletal muscle compared to kidney or brain.

In order to directly compare the fold differences in expression levels among species, the data were normalized using human levels as a target and the respective OWM as the baseline in a $2^{-\Delta\Delta Ct}$ analysis (25). Thus, the data for each ERV from the respective species are first normalized to the control gene, and then the results are further normalized by comparing human to OWM, choosing macaque or mandrill as the baseline (Fig. 2D, E, and F). Brain, as seen from all analyses, demonstrated a greatly increased expression level in humans relative to that of macaques and mandrills (20- to 60-fold) for all three HERVs, as opposed to muscle and kidney, which did not exhibit such a pronounced difference in expression. HERV-W, which was not detected in macaque 1020 muscle, was greatly reduced in expression compared to that for human muscle (Fig. 2D).

To assess the level of individual variation in ERV expression for the OWMs, two additional macaques (1022 and 1021) were tested for skeletal muscle, kidney, and brain by quantitative PCR (for HML-3 and HERV-E). In addition, to test for sex-specific expression differences, one of the two additional animals examined was male. A female macaque (1020) was arbitrarily chosen as the baseline expression, and the other two macaques were compared to it using the $2^{-\Delta\Delta Ct}$ method (25). Confirming the microarray results, HML-3 was expressed in all three OWMs with relatively minor variation in skeletal muscle (Fig. 2G). HERV-E, in contrast, was not detected in skeletal muscle for 1020 on the microarray. This was also confirmed by the quantitative PCR results, as both 1021 and 1022 demonstrated a four- to fivefold higher relative expression level compared to 1020. Kidney yielded similar results on the microarray for all three animals, although macaque 1020 exhibited somewhat higher expression than macaque 1021 or 1022. For brain, quantitative PCR demonstrated that for HERV-E, the male macaque had an almost twofold higher expression than 1020, which could explain why it was weakly detected using the microarray whereas both females were negative. Similarly, HML-3 demonstrated a twofold higher expression level in the one microarray-positive female (1021) relative to 1020.

DISCUSSION

We have employed a nonquantitative microarray system to determine the expression profiles of ERVs in nonhuman primates. This system has been successfully used to investigate the expression profiles for multiple human tissues and to determine the distribution of *pol*-containing HERVs in nonhuman primates (14, 16, 33). The results were confirmed using quantitative PCR methods.

A human-based microarray system or PCR primers based on human *pol* sequences could account for some of the differences seen between human and OWM expression. However, this would not explain the among-tissue expression differences in the same OWM. For example, in the case of mandrill, higher expression in skeletal muscle was detected for HERV-E and HERV-W compared to human muscle, excluding a general human bias in the assays used. The results remained consistent regardless of the control gene chosen or when a second unrelated human cDNA set was used for comparison. Finally, the consistent results obtained using two independent methods suggest that the observed differences are not a result of the methodology employed.

In addition to ERV sequences common to humans and OWMs, the exogenous GaLV and baboon endogenous retrovirus, both widespread among OWMs but not in humans, were included in the study. GaLV was found to be active in all OWM tissues except for mandrill liver and OWM brain, whereas BaEV was expressed in all tissues analyzed from both OWM species. GaLV entered the OWM genome via horizontal transfer from a marsupial, as suggested by the high sequence similarity between GaLV and the koala retrovirus (19). Similarly, BaEV is a cross-species jumping retroelement (38). Unlike most other ERVs, BaEV was highly active in OWM brain. The relatively high activity of both GaLV and BaEV in most tissues may reflect their ability to jump among species and their status as semiendogenized elements.

A consistent result is that more classes of HERVs, as determined by DNA microarray experiments, were observed in human brain RNA. The HERV expression level in the human brain was also higher than that observed in either OWM species as determined by element-specific quantitative PCR. The only exception is HML-10, which was detected by microarray in the brains of all species examined. Although the remaining tissues had unique expression profiles, there was no consistent up- or down-regulation relative to human tissues. In fact, while some elements demonstrated similar expression profiles among species, both OWM species differed from each other in tissue expression profile, suggesting that, overall, ERV expression is not strongly conserved among species. Individual variation in ERV expression was also observed, though it was not pronounced.

The results from brains indicate that a large part of the HERV transcriptome is generally upregulated in humans, much like general gene expression. Whether this contributes to the observed differences in morphology and cognition among primate groups remains to be determined. Of particular interest, several human diseases associated with HERVs are neurological diseases, such as multiple sclerosis (1) and schizophrenia (21). In the case of schizophrenia, however, the results are not unequivocal (14). In mice, there is also evidence of involvement of ERVs in spongiform encephalopathies (10). Thus, the higher expression of HERVs in the brain may have implications for susceptibility to neurological diseases and runs contrary to the apparent suppression of expression of even recent elements, such as GaLV in nonhuman primates.

The source of the among-primate expression variation is not known at present. Differences in expression could be the result of among-species variation in ERV copy number and genomic location, since many ERV families, such as ERV9, HERV-H, and HML-2, were retrotransposed and amplified during the speciation of OWM and hominoids. Furthermore, genome-wide epigenetic differences, such as methylation or chromatin modifications, may account for species-specific ERV activity. The differential tissue specificities of HERV expression among primates, however, may be due to differences in regulatory elements and tissue- and species-dependent availability of transcription factors. For example, control by cell-specific regulatory

proteins has been demonstrated for many human endogenous retroviral long terminal repeats (24). However, the fact that the ERV expression in OWM brains from two different species shows generally less activity than ERVs in brains of humans suggest that whatever the cause may be, it is a general effect rather than ERV specific.

ACKNOWLEDGMENT

The work in this study was supported by Bayerischer Forschungsverbund Prionen (FORPRION) grant number TUM7.

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