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The distribution of *pol* containing human endogenous retroviruses in non-human primates

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Abstract

Few human endogenous retroviruses (HERVs) have been extensively studied in non-human primates. Such investigations have demonstrated that several element classes are primate unique, contain members with important biological function, are conserved in specific primate lineages, and have in some cases expanded in copy number. We have examined multiple sub-families of all major groups of HERVs using a DNA microarray based on the reverse transcriptase (RT) domain of the viral polymerase gene (*pol*). The microarray was used to investigate the distribution of HERVs in non-human primates with particular focus on the differences between New World monkeys (NWMs) and other anthropoids. This is the first study examining most HERV families in multiple non-human primate DNAs using a uniform and sensitive method and suggests that major differences exist between primate groups. The results indicate that a major invasion and expansion of *pol* containing HERVs occurred after the platyrrhine (NWM) lineage separated from the catarrhines (Old World Monkeys and apes).

Keywords: Primates; HERV; Endogenous retrovirus; New World monkey; Old World monkey; DNA microarray; Quantitative PCR; Evolution

Introduction

HERVs are a major component of the human genome. They represent the successful colonization and expansion of retroelements in the germline of the species in which they are found. They can be divided into three broad classes each with multiple sub-families. HERV research has recently identified several elements in primates that may play a significant functional role in development. For example, syncytin-1 and syncytin-2 are both HERV envelope genes that are conserved among non-human primates (Blaise et al., 2003). In cell culture, they exhibit cell fusiogenicity, an activity which is demonstrated in most primates studied including NWMs in the case of syncytin-2. Both genes may be essential for syncytium formation in the placenta.

The lineage leading to the NWM or Platyrrhini separated from the lineage leading to the Catarrhini (Old World Monkeys, OWMs, and hominoid primates) 33 to 57 Mya (Glazko and Nei, 2003; Takahata and Satta, 1997). In some cases, retroelement evolution, that is, LINEs and SINEs, is similar among non-human primates (Boissinot et al., 2004). However, the genomic composition of HERVs differs markedly among major primate lineages. HERV-K, HERV-L, and HERV-H family distribution in non-human primates have been investigated for specific subgroups (Bénit et al., 1999; Mager and Freeman, 1995; Mayer et al., 1998; Reus et al., 2001). With the exception of HERV-L, NWMs appear to either lack or have low copy numbers of investigated HERVs. Expansion is largely restricted to catarrhine primates. Human-specific HERV integrations have also been observed indicating that the process of

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HERV expansion is still in progress among apes including humans (Barbulescu et al., 1999, 2001; Buzdin et al., 2003; Lebedev et al., 2000; Medstrand and Mager, 1998; Turner et al., 2001).

Most studies of HERV distribution in primates have used different methods, different primate species, and different HERV genes in determining presence or absence of specific element types. DNA microarrays provide a means to analyze multiple sequences rapidly and with high specificity using a single method. Recently, a microarray system using multiplex PCR with primers based on the most conserved portion of HERV subfamily RT and a HERV-specific DNA microarray spotted with portions of the RT gene of representatives of each HERV family and subfamily was developed (Seifarth et al., 2003). Using this system, we have screened multiple NWM, OWM, and hominoid DNAs for the presence or absence of specific *pol* containing HERV types. This is the largest sampling to date of non-human primates in an ERV study. The results obtained were reproducible and for specific elements confirmed using quantitative PCR and sequence analysis. It appears that the expansion of HERVs in OWM and hominoids is not restricted to specific types of elements. The general results demonstrate that NWM show a lower abundance of all HERV groups with the particular exception of class III spuma-like retroviruses (HERV-L). The data suggest that a major genome-wide evolutionary event occurred in HERV activity after the platyrrhine and catarrhine lineages separated.

Results

Class I HERVs

Fig. 1 summarizes the results of seven hybridization experiments for the class I HERVs. Members of the HERV-I family (HERV-IP, Seq 65) were detected in hominoids and OWMs and Seq 65 was detected in all groups studied though in only one NWM sample (Fig. 1). The overall copy number for HERV-I in humans is estimated at 250 copies per genome (Mager and Medstrand, 2003). For HERV-IP it is estimated to be 35 copies (Seifarth et al., 2000). In said study, a hybridization signal was detected in *Aotus* with a 700-bp RT hybridization probe. It is possible that HERV-IP has suffered deletions in NWMs and thus the smaller hybridization probe spotted on the microarray in this study does not detect it. This is not the case for Seq 65 as it was detectable in a NWM sample. Though clearly not an extremely high copy sequence in non-human primates given the weak signal detected and previous investigations of the HERV-IP subgroup (Seifarth et al., 2000), the results are consistent for this subclass of HERVs, homologs of which have been detected in such divergent phyla as fish, reptiles, and birds (Martin et al., 1997).

HERV-T (HERVS71) is known to be distributed in apes and OWMs (Haltmeier et al., 1995). This distribution was observed in this study as well. However, the element was not detected in *Cercopithecus aethiops* or *Presbytis cristata* suggesting the distribution is not continuous in OWMs. The element was not detected in any NWM tested. HERV-T is more closely related to mammalian γ-retroviruses such as MLV, GaLV, and FeLV than to other class I HERVs (Tristem, 2000; Werner et al., 1990) suggesting that HERV-T may have been acquired by cross-species transmission, that is, horizontal transfer during the diversification of the OWM lineage.

HERV-FRD (ERV-FRD, HERV-Z) were detected in Catarrhini. HERV-FRD *env* genes are known to be present in NWMs (Blaise et al., 2003, 2004). The *env* gene is also known to be highly conserved and in some cases retains active fusogenic properties. It suggests that the copy number is higher in OWMs and apes than in NWMs and/or that there is differential conservation of the *env* gene which could have an important function (Blaise et al., 2004), whereas the *pol* gene could be expendable.

HERV-W demonstrated a profile consistent with results of other studies. It has been shown previously that this element subclass is distributed throughout apes and OWMs but is absent from NWMs (Kim et al., 1999). An identical result is observed in this study showing that all apes and OWMs tested were strongly positive for HERV-W *pol* sequences and all NWMs were negative.

ERV9 distribution was found in all primates tested though there was among subclass variation. There are approximately 300 copies per genome of ERV9 related elements (Mager and Medstrand, 2003). Seq 63, ERV-9, and Seq 59 were detected in all apes and OWMs tested with the exception of *Gorilla gorilla* which did not show the presence of Seq 63. All NWMs were negative. Seq 60 had the widest distribution appearing in all apes except *G. gorilla*, all OWMs except *Mandrillus sphinx*, and in two NWMs (*Saguinus oedipus* and *Saguinus fuscicollis*). This result is consistent with the detection of ERV9 in OWMs in other studies (Widegren et al., 1996) though NWMs were never empirically tested.

Members of the HERV-ERI superfamily (ERV3, E 4-1, and Seq 32) were detected in catarrhines exclusively. This is generally consistent with previous information for OWMs (Herve et al., 2004; Shih et al., 1991). ERV3, however, was not detected in OWMs in contrast to previous findings. As the degenerate primer mix does not contain specific primers for ERV3, this may represent the favoring of related elements over ERV3 with the result that the method did not detect the element in OWMs although it is present.

It has been suggested that the HERV-ERI superfamily predates the split between Platyrrhini and Catarrhini as determined from sequence divergence estimates (Shih et al., 1991). However, none of the elements were detected in any NWM tested. This suggests that the NWM lineage lost these elements, suffered *pol* deletions, they remained at very low