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페카리 종 *Tayassu tajacu*에서 내인성 리트로 바이러스의 발견

이준헌

First Discovery of Endogenous Retroviruses in Collared Peccaries (*Tayassu Tajacu*)

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ABSTRACT

To investigate the relationship of endogenous retroviruses in peccaries and pigs, a set of degenerate primers was used in this study to amplify peccary retroviral sequences. The sequences of two putative retroviral clones showed close homology to mouse and pig retroviral sequences. The peccary endogenous retroviral sequences are significant in that they are the first such sequences reported in peccary species and repudiate old claims in the literature that peccaries do not have C-type retroviral sequences.

Introduction

Peccaries belong to the order Artiodactyla (even-toed ungulates) and to the suborder Suiformes, in which three families are listed, namely Hippopotamidae (hippos), Tayassuidae (peccaries) and Suidae (pigs) (Ruvinsky and Rothschild, 1998; Miyamoto *et al.*, 1993). They look like small hairy pigs, have a snout disc, long hair, thin legs, small eyes, and small rounded ears (Fig 1). Fossil records of Tayassuidae have been found in Eurasia and even in Africa suggesting

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that they were separated from Suidae not later than in the Oligocene era (30 million years before present, MYBP). However, modern peccary species live only in the Americas (Ducrocq, 1994; Hendey, 1976). The family Tayassuidae seems to have originated in South East Asia and then migrated into the New World, because the most morphologically primitive fossils are found in Thailand (Ducrocq, 1994).

The C-type retroviruses are widespread as both endogenous and exogenous agents within mammalian species including humans (Tristem *et al.*, 1996b). Tristem *et al.* (1996b) designed degenerate primers based on conserved motifs in the protease and reverse transcriptase gene of Murine leukemia viruses (MLVs) for amplifying retroviral sequences in a variety of mammalian species. Other degenerate primers were also designed based on conserved motifs of the retroviral protease and reverse transcriptase protein for the same purpose (Tristem *et al.*, 1996b). These primers are capable of amplifying retroviruses and long terminal repeats (LTR) retrotransposons in wide range of vertebrate taxa including mammals, reptiles, amphibians and fish (Herniou *et al.*, 1998; Martin *et al.*, 1997; Tristem, 1996; Tristem *et al.*, 1996a).

Based on very early work of DNA hybridization, it has been claimed that peccaries do not contain virogene sequences related to the pig and mouse type C-virus (Benveniste and Todaro, 1975). The c-type endogenous retroviruses in pigs have become a focus of concern in relation to the xenotransplantation. Pigs have about 50 known PERVs (Porcine Endogenous Retroviruses) and their integration sites in the genome are different among breeds of pigs (Lee *et al.*, 2002). If peccaries does not have endogenous retroviruses as presented in previous research (Benveniste and Todaro, 1975), then this species might be very valuable resources for the transplantation organs and tissues for humans. The aim of the work reported here is to investigate the existence and nature of endogenous retroviral sequences in the Collared peccary using the degenerate primers of Tristem *et al.* (1996b).

Fig. 1. A Collared Peccary (*Tayassu tajacu*) (Courtesy of Jaime Gongora).

Materials and Methods

1. The peccary genomic DNA samples

Five Collared peccary genomic DNAs were supplied by Mr. Jaime Gongora. They were collected in 1999 at Santa Cruz Zoo which is located near Bogota, Colombia.

2. Primers

The degenerate primers are based on two motifs conserved in a representative set of C-type retroviruses, especially in MLVs. Retroviral sequences have been selectively and specifically amplified in many different mammalian species using these primers (Tristem, 1996; Tristem *et al.*, 1996b). The primer sequences are 5'-(C/T)TI(T/G)TIGA(T/C)ACIGGIGCI(G/C)A-3' for the forward primer and 5'-AGIAGGTC(A/G)TCIAC(A/G)TA(C/G)TG-3' for the reverse primer. Note that I in the primer sequences stands for inosine.

3. PCR (Polymerase Chain Reaction)

PCR was carried out in a 25 ul volume with approximately 100 ng of template genomic DNA, 0.4 mM of each primer, 600 uM of each dNTP, 1 mM $MgCl_2$, 10x PCR buffer, and 2.5 units of *Taq* polymerase. This mixture was overlaid with one drop of mineral oil. Amplification was performed in a PTC-100 Programmable Thermal Controller (MJ Research Inc., USA) for 45 cycles with denaturation for 1 minute at 95°C, annealing for 30 seconds at 47°C and extension for 1 minute at 72°C. The final extension was for 20 minutes at 72°C.

4. Cloning of the PCR products

The peccary retroviral PCR products were cloned into the pCR2.1-TOPO plasmid vector (Invitrogen, USA) followed by the manufacturer's instruction.

5. Sequencing of the clone inserts

A *SequiTherm* EXCEL™ Long-Read™ DNA sequencing kit (Epicentre, USA) and Li-Cor sequencer (Model 4200, Li-Cor Inc., USA) were used to read peccary insert sequences, using two M13 vector primers (Li-Cor Inc.) labeled with different dyes. Sequences were analyzed using Base ImageIR™ software version 4.1 (Li-Cor Inc.).

Results and Discussion

1. PCR amplication for retroviral sequences in Collared peccary

The degenerate primer pairs were tested in five different collared peccary genomic DNAs. The agarose gel shows that the PCR reactions produced two bands in some peccary samples (Fig. 2). The 434 bp product was common to all templates but the 840 bp products amplified in only two peccary DNA samples. The 840 bp PCR product was found to amplify variably depending upon the PCR conditions (data not shown). Most mammalian species, including pig, gave only a 950 bp product with these degenerate primers (data not shown) as described by Tristem *et al.* (1996b). Therefore, neither the 434 nor 840 bp PCR products were expected as correctly amplified retroviral fragments.

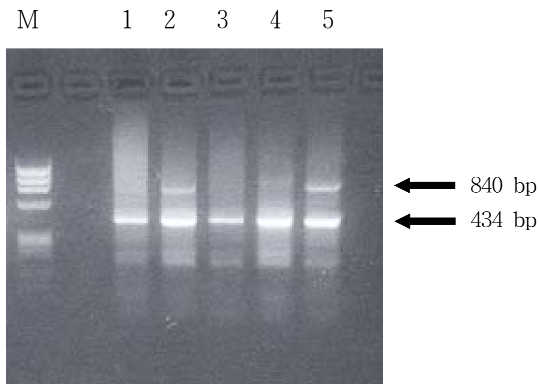

Fig. 2. PCR products of five Collared peccaries (nos 1 to 5) amplified by the degenerate primers. A 434 bp product is common to all amplifications but the 840 bp product occurs in only two samples (lane two and five). Marker (M) is ØX174/*Hae*III size standard marker (Promega, USA).

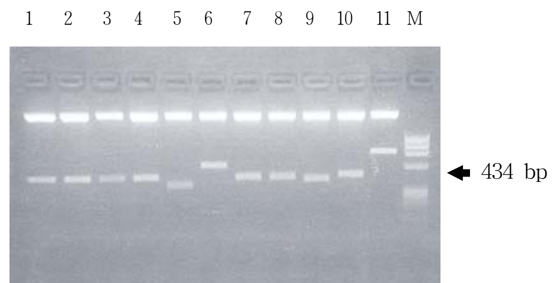

Fig. 3. *Eco*RI restriction digestion of peccary clones. Lanes 1 to 10 (designated as clones S1 to S10) are the putatively 434 bp insert clones. Lane 11 (designated as clone L1) is the putative 840 bp insert clone. M is ØX174/*Hae*III size standard marker (Promega).

2. Sequences of the Peccary retroviral clones

After cloning, *Eco*RI digestion was used to release the insert from the vector to confirm its size. The recognition site of this enzyme is located in the multi-cloning site and can be used to excise the DNA insert. Fig 3 shows the *Eco*RI restriction enzyme digestion pattern of a series of peccary clones derived from the PCR product of lane number 5 in Fig. 2. The attempt to clone the 434 bp PCR products gave inserts of varying size (Fig 3. lane 1 to 10). Only one clone with the 840 bp insert was analysed (Fig. 3. lane 11). The clones from the putatively 434 bp PCR product of lane 5 Fig 2 are designated as clones S1 to S10 based on their order in Fig 3. The 840 bp insert clone (Fig. 3. lane 11) is designated as clone L1.

Six peccary putative 434 bp clones (clone S1, S2, S5, S6, S9, S10) which were selected to cover all insert sizes and the one 840 bp clone (clone L1) were fully sequenced. The FASTA sequence search results showed that clones S1 and S2 were the only clones with strong matches (expectation value with 1.5 X e^{-26}) to retroviral sequences (Fig. 4). They were both well matched with known murine leukemia virus sequences in GenBank, demonstrating that at least some of the putative 434 bp PCR products are from endogenous retroviral sequences in Collared peccary. Interestingly, the FASTA results show that the peccary retroviral sequences are better matched with mouse sequences than with pig retroviruses, although the match with pig retroviral sequence is also excellent (data not shown).

The alignments of the two peccary retroviral

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MULV13893 Murine leukemia virus RNA for gag-pol-env pol (8282 nt)
 initn: 567 init1: 403 opt: 542 Z-score: 584.2 expect() 1.5e-26
 63.975% identity in 322 nt overlap

                                          10        20        30
Clone S2                                 CTGGTGGACACGGGGGCGCAACATTCGGTC
                                         :: ::::: :: ::::: ::::: :: ::
  MULV13 CTCAAAGTCGGGGGGCAACCCGTCACCTTCCTAGTGGATACTGGGCCCAACACTCCGTG
             2280      2290      2300      2310      2320      2330

                   40        50        60        70        80        90
Clone S2 TTAGTCAAATCTCATGGAAAAATCTCTGACAAATCCTCCTGGGTCCAAGGGGCTACCGGA
           :   : ::  :: ::::    :   :::::: ::  :::::::::::::::::: :::
  MULV13 CTGACCCAAAATCCTGGACCCCTAAGTGACAAGTCTGCCTGGGTCCAAGGGGCTACTGGA
             2340      2350      2360      2370      2380      2390

                  100       110       120       130       140       150
Clone S2 GTCAGACGTTACCCCTGGACTACTCAGAGAACTGTGGACTTGGGTACCGGGAAAGTGACT
           :  :  :: :: : :::::: ::  :  :    ::: :: : :  ::::: :: :: ::
  MULV13 GGAAAGCGATATCGCTGGACCACGGATCGCCGAGTGCACCTAGCCACCGGTAAGGTCACC
             2400      2410      2420      2430      2440      2450

                  160       170       180       190       200       210
Clone S2 CACTCCTTCTTAGTAATCCCCGATAGCCCCTGCCCTTTACTGGGGAGAGACTTATTCACC
           ::::: ::: :     : :: ::  ::::::  ::: : :: :: ::::: ::  : ::
  MULV13 CACTCTTTCCTCCATGTACCAGACTGCCCCTATCCTCTGCTAGGAAGAGATTTGCTGACT
             2460      2470      2480      2490      2500      2510

                  220       230          240       250       260
Clone S2 AAGATGGGAGTGCAAATTCACTTT---CGACTAGGAGAACCAATTGTAACCGGACCACAA
           ::  :   ::  ::::::::::::    ::  ::::: :   ::::    ::::::
  MULV13 AAATTAAAAGCCCAAATTCACTTTGAGGGATCAGGAGCTCAGGTTGT---GGGACCAATG
             2520      2530      2540      2550      2560         2570

               270       280       290       300       310       320
Clone S2 GAACTCCCTATATCGGTGCTTACCCTAAGATTAGACGATGAATACCGACTTCCACAAGGA
           : ::  ::  :    ::::: :::::::   :::: ::::: :: :: :: :
  MULV13 GGACAGCCCCTGCAAGTGCTGACCCTAAACATAGAAGATGAGTATCGGCTACATGAGACC
                2580      2590      2600      2610      2620      2630

               330       340       350       360       370       380
Clone S2 TTCAACAATTCACCCACCCTGTTTGATGAAGCCCTCCATGGGGACCTCAGTGAGTATCGA

  MULV13 TCTAAAGGGCCAGATGTGCCTCTAGGGTCCACATGGCTCTCTGATTTTCCCCAGGCCTGG
                2640      2650      2660      2670      2680      2690
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Fig. 4. Sequence alignment of the peccary endogenous retroviral sequences (clone S2) with the murine leukemia virus sequence (GenBank accession number MULV13893). They show 64 % sequence identity between the two species.

sequences show that there are eight base-pair differences between these two clones. The 0.4 % error rate for the Li-Cor sequencer has been calculated elsewhere (Lee *at al.*, 2002) from analysis of very long (~1.8 kb) sequence reads from porcine endogenous retrovirus clones. However, most of these sequencing errors are located at the end of the sequences, after 600~800 bp from the beginning. These shorter peccary retroviral sequences are fully overlapped in both forward and reverse directions and thus are confirmed double pass sequences. Thus it can be assumed that these peccary retroviral sequences are truly different and represent multiple copies, as in other species.

The sequence search results for the other insert clones (clone S5, S6, S9, S10, L1) show that these PCR products are not derived from the endogenous retrovirus. Therefore these were come from the artifactual PCR amplication products due to the degenerate primers.

In conclusion, two clones (S1 and S2) show close homology to mouse and pig retroviral sequences. These are the first retroviral sequences identified in peccary. They clearly contradict the observations of Benveniste and Todaro (1975) based on Southern hybridization that pigs only, and not peccaries, contain C-type retroviruses which they claimed were derived from an invasion of the pig genome by murine retroviruses after the split of the Suidae and Tayassuidae lineages. The result presented here clearly shows that endogenous retroviral sequences are present in the peccary genome and that these sequences are very similar to mouse retroviral sequences.

적요

본 연구는 페카리와 돼지간의 내인성 리트로 바이러스의 연관성을 조사하기 위하여 실시되었으며 페카리의 리트로 바이러스의 DNA 염기서열을 알기위해 degenerate primers를 이용하였다. 두개의 리트로 바이러스의 클론이 이 연구에 의해 만들어졌으며 DNA 염기서열을 분석하여 본 결과 기존에 알려진 쥐와 돼지의 리트로 바이러스 염기서열과 높은 상동성을 보였다. 이 페카리 리트로 바이러스는 페카리 종에 있어서 처음으로 밝혀진 내인성 리트로 바이러스인 동시에 페카리 종은 C형 리트로 바이러스 염기서열을 가지고 있지 않다는 기존의 연구결과와 상반된 것으로 그 중요성이 있다.

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