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Endogenous JSRV-like proviruses in domestic cattle: Analysis of sequences and transcripts

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Abstract

Jaagsiekte retrovirus is an exogenous (exJSRV) beta-retrovirus with a simple genome. It causes lower airway epithelial cell tumors in small ruminants. Endogenous (enJSRV) counterparts of exJSRV are present in different copy numbers in numerous *Bovidae* family members.

This work has focused on enJSRV in Simmental (Germany) and Limousine (France) beef breeds of domestic cattle and domestic goat. Of the enJSRV sequences in cattle, the *orf-x* sequences were about 99% identical, the LTR sequences were about 97% identical and the *env* sequences were nearly 95% identical to the corresponding endogenous sequences in sheep. A significant polymorphism of the proviral sequences between the cattle breeds was noted. Clonal analyses of the amplicons suggest two enJSRV proviruses in cattle genome. The endogenous sequences revealed in goat were closer to enzootic nasal tumor virus (ENTV) from goat rather than to enJSRV from sheep.

The expression of enJSRV in cattle was partial (*env* only) and detected exclusively in bone marrow.

Keywords: Jaagsiekte sheep retrovirus (JSRV); Endogenous retroviruses; Domestic cattle; Goat; Sequences; Body compartments; Transcripts

Introduction

Endogenous retroviruses (ERV) and retroviral elements have been found in the genome of all examined vertebrates (Weiss, 2006). Most of the known ERV sequences are incomplete, largely deleted and/or interrupted by stop-codons. However, complete ERV have been described and some of them are capable of forming virus-like particles (vlp) (Patience, 1999). ERV have not been examined in detail in animals other than various laboratory species.

The pathogenicity of ERV is currently considered to have minimal biological significance. However, this is still under investigation. Besides the other possibilities, a recombination between ERV and exogenous retroviruses may create "new" exogenous retroviruses. ERV might be beneficial during embryogenesis, offering protection against an infection with related exogenous retroviruses (Kalter et al., 1975; Palmarini et al., 2004).

The sheep pulmonary adenomatosis (SPA) or the ovine pulmonary adenocarcinoma (OPA) is a contagious lung cancer caused by an exogenous Jaagsiekte sheep retrovirus (exJSRV) (Sharp, 1987; DeMartini and York, 1997; Palmarini and Fan, 2001; Suau et al., 2006). The exogenous enzootic nasal tumor virus (ENTV) is closely related to JSRV, but causes nasal tumors in small ruminants (De las Heras et al., 1991; Ortin et al., 2003). The incidence of OPA in affected animals is nearly 2% to 5% (Sharp and DeMartini, 2003). In fact, a minority of JSRV-infected animals develops a clinical disease during their commercial lifespan (Caporale et al., 2005).

JSRV is a chimera of simian type D retrovirus (SRV) and mouse mammary tumor virus (MMTV) with *gag*, *pro* and *pol* genes that are related to those of SRV-3 (Mason-Pfizer monkey virus), and an *env* gene is closer to that of MMTV (York et al., 1992). JSRV has a "simple" genetic organization with classical retroviral genes *gag, pro, pol* and *env*. It has an additional ORF (*orf*-x) of unknown function in *pol*. The *orf-x* gene, the most

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conserved part of known JSRV isolates, is not linked to transformation. JSRV is an oncogenic virus without a discrete oncogene, but it has a short peptide (Y-X-X-M) in ENV that acts as an oncoprotein (Maeda et al., 2001; Alberti et al., 2002; Fan et al., 2003; Miller et al., 2004; Wootton et al., 2005; Leroux et al., 2007). However, an insertional mutagenesis leading to transformation could not be excluded (Cousens et al., 2004).

A human infection with exJSRV-like viruses could not be excluded (De las Heras et al., 2000; Morozov et al., 2004). On the other hand, the exJSRV has not been convincingly linked to human pulmonary adenocarcinoma (Yousem et al., 2001; Hiatt and Highsmith, 2002).

The exJSRV is very similar to a family of enJSRV in sheep and goats (York et al., 1992; DeMartini et al., 2003; Palmarini et al., 2004). The amino acid sequences of exJSRV and enJSRV are between 90% and 98% identical, depending on the gene and the geographical origin of examined animals. Three short variable regions (VR), two in *gag* (VR1, VR2), one in *env* (VR3), are principal "divergence" markers of enJSRV and exJSRV (Bai et al., 1996; Palmarini et al., 2004).

The expression of enJSRV in sheep is progesterone dependent and high levels of provirus expression occur in endometrial epithelia of the ovine uterus and ovine fetus (Palmarini et al., 2001). A less enJSRV expression occurs in the lung, kidney, bone marrow, spleen and in various other tissues (Palmarini et al., 1996). The cellular receptor for exJSRV is (HYAL-2), a membrane protein conserved across various species (Rai et al., 2001).

An extensive search in mammals for the enJSRV genomes by Southern blot hybridization identified proviruses in the families of the *Artiodactyla* order and showed that the highest load of enJSRV sequences occurred in the *Caprina* subfamily (Hecht et al., 1996). The ovine and domestic goat (*Capra hircus*) genomes contain 15 to 20 copies of enJSRV. Of three enJSRV from sheep that were sequenced, two were interrupted by internal stop codons and the third was nearly intact (Palmarini et al., 2000). Little is known about enJSRV in other "positive" animals. The genome of domestic cattle (*Bos taurus*, *Bovinae* subfamily) is predicted to have one to three enJSRV-related proviruses (Hecht et al., 1996). However, neither proviruses in cattle, nor putative expression have been characterized.

In this report, we analyzed enJSRV-related proviruses in two European cattle beef breeds Simmental (Germany) and Limousine (France). The expression of the enJSRV in cattle was examined in body compartments. We also analyzed enJSRV-like sequences and expression profile in domestic goat from Germany.

Of the enJSRV sequences identified in cattle, the sequence of *orf-x* was the most similar (98% to 99%) to the enJSRV sequences in sheep. The LTR sequences were less similar. Their sequences were about 97% identical to the known enJSRV sequences in sheep. The LTR amplicons from cattle formed an independent cluster of sequences in the phylogenetic analysis, and a clonal analysis of the LTR amplicons demonstrated two close types of sequences in examined animal. The bovine enJSRV-like *env* sequences were the least similar (94%–95%) to corresponding enJSRV sequences in sheep. A notable polymorphism was evident when the *orf-x* and *env* sequences from animals belonging to different breeds were compared in between each other. The analyses of body compartments of cattle resulted in a detection of the *env* transcripts, exclusively in bone marrow, while no *orf-x* transcripts were detected. We showed that the endogenous proviral *orf-x* and *env* sequences revealed in goat were more similar to the ENTV from goat rather than to the enJSRV from sheep. Only *env* transcripts were detected in bone marrow of the goat.

Results

Southern hybridization analysis

The data, regarding enJSRV-like proviruses in domestic cattle, came exclusively from genomic Southern hybridization analysis. Under high stringency washing conditions, capsid (CA) and surface (SU) subgenomic probes detected one to three proviruses in the genome of domestic cattle (Hecht et al., 1996).

We examined genomic DNA from Simmental cow by Southern hybridization using enJSRV *orf-x* subgenomic probe. Besides genomic DNA from Simmental cow, we examined genomic DNA from sheep (positive control), pig, African green monkey and an healthy blood donor. DNA specimens were digested with *Pvu*II. There are several sites for restriction endonuclease *Pvu*II in exJSRV (accession no. AF105220) and enJSRV (accession no. AF153615) genomes (Fig. 1A). Two of them are flanking a central part of the genome (positions 2986 and 5583, accession no. AF105220). Thus, in case of *pol* (including *orf-x*) integrity, the resulting cleavage fragment would be of nearly 2.6 kb. Southern blots was hybridized with a DIG-labeled JSRV *orf*-x subgenomic probe and washed under conditions of high stringency. The hybridization revealed one band in DNA from cow and two bands in DNA from sheep (Fig. 1B). The upper band (2.6 kb) corresponding to the intact *pol* fragment was identical in both samples. The lower band (2.3 kb) was revealed only in sheep DNA and that was likely a truncated *pol* fragment (Fig. 1B). The identification of 2.6 kb proviral fragment after digestion with *Pvu*II suggests an identity of the physical maps of the region in sheep and cattle proviruses. It is likely that the *Eco*R1 site (in pol) in provirus(es) from cattle was also retained. However, that was not proved by double digestion with *Bam*H1 or *Pvu*II.

No enJSRV *orf-x* sequences in pig, human and African green monkey genomes were detected by genomic Southern hybridization.

EnJSRV provirus in domestic cattle and goat

Further analyses of enJSRV-like proviruses in cattle (and goat) were done by nested PCR with a set of JSRV-specific primers (Table 1).

The most conserved part of the JSRV genome is considered to be *orf-x* (Rosati et al., 2000). Since it was impossible to predict the enJSRV-like sequences in cattle, we initially focused on that part of the genome in our screening for proviruses.