Placenta 32 (2011) 493–499

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Expression of endogenous retrovirus-like transcripts in bovine trophoblastic cells

K. Koshi [a,b], K. Ushizawa [c], K. Kizaki [a,b], T. Takahashi [c], K. Hashizume [a,b,*]

[a] Laboratory of Veterinary Physiology, Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan
[b] United Graduate School of Veterinary Science, Gifu University, Japan
[c] Department of Developmental Biology, National Institute of Agrobiological Sciences, Ikenodai 2, Tsukuba, Ibaraki 305-8602, Japan

ARTICLE INFO

Article history:
Accepted 4 April 2011

Keywords:
Endogenous retrovirus
ERV-derived gene
Trophoblastic cells
Binucleate cells

ABSTRACT

Endogenous retrovirus envelope elements are considered to participate in trophoblastic cell fusion and multinucleate cell formation in humans, mice, and sheep. However, there is limited information about their roles in the ruminant placenta.

Objectives: We explore and identify the endogenous retrovirus envelope element genes expressed in bovine trophoblasts.

Methods: The NCBI UniGene database (Build #97 *Bos taurus*) was screened by *in silico* analysis. After cloning endogenous retrovirus envelope element-like transcript (ERVE), expression profiles were analyzed with quantitative RT-PCR and *in situ* hybrizaidation.

Results: Two UniGene clusters, UniGene ID: Bt.68042 and Bt.85243, were detected, and ERVE-A gene was cloned. Weak expression of this gene was first detected on Day 20 of gestation, and the intensity of its expression increased up to Day 70 of gestation. The intensity of its expression was maintained throughout gestation in the placenta, and its specific expression in trophoblastic binucleate cells was confirmed by *in situ* hybridization.

Conclusions: bERVE-A has a similar sequence to human syncytin-1, although it lacks an intact envelope sequence, and is specifically expressed in binucleate cells. This is the first evidence that endogenous retrovirus envelope element genes are expressed in bovine binucleate cells.

1. Introduction

The placenta is the first organ that promotes the survival and growth of embryos in mammals. In cattle, it is known that two specific trophoblastic cell types, binucleate cells (BNC) and mononucleate cells (TMC), play a crucial role in cattle placentation [1]. Both produce cell specific molecules associated with the establishment and maintenance of pregnancy. BNC express placental lactogen (bPL; gene name: *bCSH1*) [2–4], prolactin-related proteins (PRP) [5], and pregnancy-associated glycoproteins (PAG) [6], and TMC express interferon-tau (*IFNT*) [7] and secrete the Ly-6 domain 1 protein [8]. In mice, which have a hemochorial placenta, the trophoblastic cell lineage has been investigated, and a theory regarding the role of stem cells in placental trophoblast development has been constructed [9,10]. Rodent trophoblasts include several specialized cell subtypes, including spongiotrophoblasts, trophoblast giant cells, and glycogen trophoblast cells [11–13]. However, the origin and fate of trophoblastic cells have not been clarified in ruminants, and the roles played by the bovine trophoblastic cell lineage are disputed. Although bPL (*bCSH1*) is a well-known marker of BNC, the BNC population is comprised of at least two different cell types: those expressing PL and those that do not [14–17]. Therefore, various types of trophoblastic cell might exist in the bovine placenta, including BNC with different characteristics, as is found in mice [18].

Recently, endogenous retrovirus (ERV) genes known as endogenous Jaagsiekte sheep retroviruses (enJSRV) have been reported to be expressed in the sheep placenta, and they may play specific developmental and morphogenic roles in the peri-implantation ovine conceptus [19]. ERV are present in all vertebrates and are thought to arise from ancient infections of the host's germline by exogenous retroviruses (*gag*-, *pol*-, and *env*-related regions bordered by two long terminal repeats). However, although they are thought of as junk DNA, intact *env* genes are expressed in the placental trophoblasts of some species [20–23]. In humans, two *env* were identified, *syncytin-1* and *syncytin-2*, which induce cell–cell fusion to produce syncytiotrophoblasts [22,24–28]. In sheep, enJSRV is suggested to play a similar role in the trophoblastic cell lineage [19].

Abbreviations: ERVE, endogenous retrovirus envelope element-like transcripts; ERV, endogenous retrovirus; BNC, binucleate cell; TMC, mononucleate cell.

* Corresponding author. Laboratory of Veterinary Physiology, Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan. Fax: +81 19 621 6210.
E-mail address: kazuha@iwate-u.ac.jp (K. Hashizume).

0143-4004/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.placenta.2011.04.002

Table 1
Tissue collection schedule.

Group	Collection period	No. of animals
Estrous cycle		
Stage I–II	Days 1–10	5
Stage III	Days 11–17	6
Stage IV	Days 18–20	11
Gestation		
Day 20	Day 17, 18, 19, 21	4
Day 30	Day 27, 30[a], 35, 35, 37	5
Day 70	Day 64, 64, 65, 78[a]	4
Day 120	Day 120[a], 120, 120, 144, 156	5
Day 210	Day 174[a], 224, 245, 259	4

qRT-PCR: Quantitative real-time RT-PCR.
[a] Used for the *in situ* hybridization shown in Fig. 4.

In a previous study, we found a candidate for a similar gene in cattle [29]. These results suggest that ERV genes play an important role in the development of the placenta and the trophoblastic cell lineage; however, the details of their expression and functions remain unclear.

The purpose of this study is to comprehensively examine the ERV-derived gene candidates present in the bovine placenta. First, we examined bovine ERV-derived genes that display similarity to human syncytin-1 in the NCBI UniGene database (Build #97 *Bos taurus*) using *in silico* analysis. Second, after determining the candidate bovine ERV genes, we examined their expression and localization in the bovine endometrium and placenta.

2. Materials and methods

2.1. Animals and tissue collection

Tissues were collected from Japanese Black cows at different functional stages of the estrous cycle and gestation and subjected to Northern blotting, cDNA cloning, quantitative real-time RT-PCR (qRT-PCR), and *in situ* hybridization according to the objectives of this study. The physiological status of the estrous cycle and gestation were defined using ovarian morphological definitions and the day of gestation (Day of AI = Day 0 of gestation) (Table 1), as described in previous reports [30]. Placentomal and endometrial tissues including caruncular (CAR), intercaruncular (ICAR), cotyledonary (COT), and intercotyledonary (ICOT) tissues were collected separately and used for total RNA extraction. Histological studies were performed using the endometrium and the placentome on Day 30, Day 70, Day 120, and Day 210 of gestation. These tissues were fixed in 4% paraformaldehyde-phosphate buffered saline (PBS, pH 7.4) and stored at 4 °C. Some pregnant uteri were perfused with 4% (w/v) paraformaldehyde PBS, and all tissues used for the histological examinations were handled according to the procedures described in a previous report [31]. cDNA cloning and Northern blot analysis were performed using Day 210 COT tissue and stage I–II endometrial tissue. qRT-PCR was performed using cyclic endometrial tissues, and fetal and placentomal tissues during gestation. Other somatic tissues including the skeletal muscle, testes, and ovaries were used for gene expression comparisons. All tissues were snap frozen in liquid nitrogen immediately after collection and stored at −80 °C until the RNA extraction. All animal procedures were carried out in accordance with the guidelines and ethics set out by the Animal Care and Use Committee of Iwate University and the National Institute of Agrobiological Sciences, Japan.

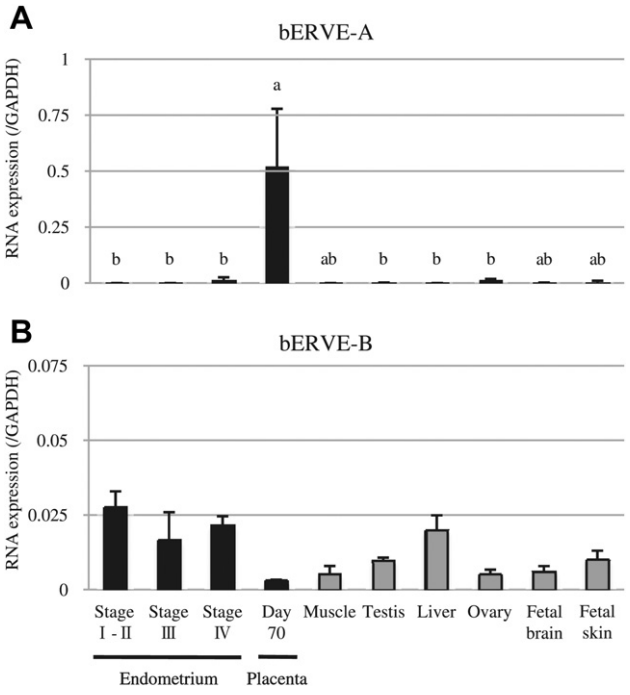

Fig. 1. Gene expression analysis of bERVE-A and -B in bovine tissues. The expression of bERVE mRNA in various bovine tissues, including the ovaries, liver, muscle, testes, fetal brain, and fetal skin was analyzed by qRT-PCR. Cotyledonary tissue at Day 70 of gestation was used as a bovine placental sample. (A) bERVE-A mRNA, (B) bERVE-B mRNA. Bar graph showing the mean ± SEM. Data labeled with different letters are significantly different from each other ($p < 0.05$).

2.2. In silico analysis of bovine ERV genes

We searched for bovine ERV genes on the NCBI Map Viewer website [http://www.ncbi.nlm.nih.gov/mapview/] using a syncytin sequence and found two candidates sequences, UniGene ID: Bt.68042 and Bt.85243, which were then subjected to further analysis; i.e., cloning, sequence analysis, expression, localization, and an analysis of their genomic status.

2.3. RNA extraction and RT-PCR

Total RNA was individually isolated using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Genomic DNA was removed using DNase and the Turbo DNA Free Kit (Ambion, Austin, TX, USA). Two micrograms of total RNA were reverse transcribed into cDNA using random primers and a high capacity reverse transcriptase kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The RT cycle comprised 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 s in a thermal cycler, and the resultant cDNA was stored at −20 °C. The PCR primers were designed using the Primer-3 software [http://primer3.sourceforge.net/] based on

Table 2
Oligonucleotide primers used for RT-PCR analysis, RACE analysis, and qRT-PCR analysis.

Gene	Primer	Sequence	Position	Use
Bt.68042	Forward	TTCCTCAAAGAAGAAGAGGTAGAACAA	703277–703304	RT-PCR
(GenBank ID: NW_001493691.1)	Reverse	GGGTCCAAATAAGAGGAATAGAATGAT	703775–703748	
Bt.85243	Forward	GAACTTAATGAGGATATGGAGCAGGTA	3100208–3100181	RT-PCR
(GenBank ID: NW_001493674.2)	Reverse	GACGTTTTGGGTAATCTTTAGTTGAGA	3099429–3099456	
bERVE-A	5′-RACE	AGGTCCAGGGCTCGCCTATTTTGAAGAG	703554–703526	RACE
(NW_001493691.1)	3′-RACE	GACGAGCTGTCATAGCCCCTCTCCTGAT	703338–703366	
bERVE-A	Forward	GGATCTGACGGGAGACACAAA	703321–703301	qRT-PCR
(NW_001493691.1)	Reverse	CACCAATCCGGGAATCTTCA	703260–703281	
bERVE-B	Forward	GGCCCAAGCACTCCTTCAT	3100010–3099992	qRT-PCR
(NW_001493674.2)	Reverse	CGCCCTTTTTCCCATTTCTT	3099954–3099973	
GAPDH	Forward	AAGGCCATCACCATCTTCCA	280–300	qRT-PCR
(GenBank ID: NM_001034034)	Reverse	CCACCACATACTCAGCACCAGCAT	355–331	