Assignment¹ of the protein-tyrosine phosphatase beta gene (PTPRB) to cattle chromosome 5q23→q24 by in situ hybridization and somatic cell panel analysis

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¹ To our knowledge this is the first time this gene has been mapped in cattle.

Rationale and significance

Protein tyrosine phosphatases, together with protein tyrosine kinases, regulate the tyrosine phosphorylation that controls cell activities and proliferation. PTPRB contains an extracellular domain, a single transmembrane segment and one intracytoplasmic catalytic domain, hence it belongs to receptor type protein tyrosine phosphatases.

The extracellular region of PTPRB is composed of multiple fibronectin type-3 repeats, which was shown to interact with neuronal receptor and cell adhesion molecules, such as contactin (Rios et al., 2000) and tenascin C (Adamsky et al., 2001, 2003). Expression analysis implicate the roles of PTPRB in cell adhesion, neurite growth, and neuronal differentiation (Nagata et al., 2003). In cattle cell lines, protein tyrosine phosphatases and their substrates were suggested to regulate adherens junctional integrity, the movement of macromolecules and cells through the endothelial paracellular pathway, and capillary tube stability, thus possibly being involved in the immune response (Young et al., 2003).

PTPRB was also found to interact with ATP-gated ion channels in the plasma membrane, and may regulate channels by altering their tyrosine phosphorylation status (Ratcliffe et al., 2000; Kim et al., 2001). It provides a component in cell-cell and

cell-extracellular matrix signaling events (Garwood et al., 2003).

In human PTPRB was mapped previously to HSA12q15→q21 by FISH (Harder et al., 1992).

Materials and methods

Isolation and characterization of bovine PTPRB gene

Comparative primers were designed from the human PTPRB sequence and tested on bovine genomic DNA. A 153-bp fragment was achieved and sequenced. The sequence displays a homology of 93 % with human PTPRB (X54131; Identities = 134/144, Expect = 8e-51). The following primers designed from the bovine sequences were used for PCR amplification and BAC screening:

PTPRB F: 5'-CTGCCCTCCTACCTGGAATA-3'

PTPRB R: 5'-ACATTTTCCACCCAGGCTCT-3'

Somatic cell panel analysis

A bovine hamster somatic cell hybrid panel (Womack and Moll, 1986) was screened using the primers described above. PCR conditions were 95 °C for 30 s, 58 °C for 45 s and 72 °C for 45 s repeated in 35 cycles with an initial denaturation of DNA for 2 min at 95 °C and a final extension step at 72 °C for 10 min. Statistical analysis for identification of synteny was done as described previously (Chevalet and Corpet, 1986).

Fluorescence in situ hybridization (FISH)

The described primer set was used for PCR screening of the bovine BAC library BBI_750 (Zhu et al., 1999). Standard PCR was performed as described above. The identified BAC was labeled with Biotin-16-dUTP and used for FISH on photographed GTG-banded cattle chromosomes. The hybridization followed the standard protocol (Pinkel et al., 1986). The analysis of FITC fluorescence signals was done on propidium iodide stained chromosomes.

Probe name: BBI_B750 A10307

Probe type: Bovine genomic DNA containing BAC clone

Insert size: ~ 100 kb

Vector: pBACe3.6 (BAC)

Proof of authenticity: PCR, DNA sequencing, BLAST

Gene reference: X54131

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Fig. 1 . Assignment of PTPRB to BTA5 by FISH. (left) Metaphase after FISH with PTPRB specific BAC clone; (right) GTG banded metaphase. Arrows indicate FISH signals and BTA5, respectively.

Results

Mapping data

Most precise location: BTA5q23

Nucleotide position in human chromosome reference sequence: 70663361–70663512

Number of cells examined: 22

Number of cells with specific signal:

1 (0), 2 (0), 3 (7), 4 (15) chromatids per cell

Location of background signals: none observed

Mapping by FL

Number of chromosomes examined: 38

Mean location: 0.44

Bands encompassed: BTA5q23

Range: BTA5q23 → q24

Standard deviation: ± 0.021

Using the PTPRB primers, we detected a 153-bp bovine-specific band in 13 of the 31 hybrid cell lines. The data vector obtained was: 0000011100011101011110000001100. These results allowed the assignment of PTPRB to cattle chromosome 5 with a concordance value of 0.94 confirming our FISH mapping results.

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