Assignment of syndecan 2 (SDC2)¹ gene to cattle chromosome band 14q22 and thymus high mobility group box protein TOX (TOX)² gene to cattle chromosome band 14q17→q18 by in situ hybridization

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

² This is a more precise localization of a gene previously mapped to BTA14 by Goldammer et al. (2002).

Rationale and significance

Syndecan 2 gene codes for a transmembrane heparan sulfate proteoglycan protein that has an unclear function at the cell surface. The tumorigenic activity of SDC2 in colon carcinoma cell behavior and its influence on the regulation of adhesion and proliferation in colon carcinoma cell has been described (Park et al., 2002). TOX represents a thymus high mobility group box protein that is involved in the regulation of thymocyte selection (Wilkinson et al., 2002). Human SDC2 (alternate HSPG) was assigned on chromosome band HSA8q22→q23 (Marynen et al., 1989) and human TOX (alternate KIAA0808) was mapped on position 325.53 cR3000 in the Genebridge 4 RH-Map (Gyapay et al., 1996), which corresponds to chromosome region HSA8q11.23. We report here the localization of SDC2 and TOX on cattle chromosome BTA14 by fluorescence in situ hybridization.

Materials and methods

Isolation and characterization of bovine SDC2 and TOX genes

Bovine SDC2 and TOX partial cDNA sequences were isolated from liver and intestine, respectively, using mRNA reverse transcription. The sequences display a significant homology of higher than 90 % with the corresponding human gene sequences. For SDC2, 281 bp in length, a homology of 92 % with human SDC2 (XM_040582) was found (Identities = 223/240, Expect = 3e-94) whereas the blast search for TOX, 224 bp in length, resulted in 95 % homology with human TOX (NM_014729, Identities = 204/213, Expect = 6e-92).

The following primers designed from the bovine sequences were used for PCR amplification:

SDC2 F: 5'-AAAATCAGTTTTTGTCTTCA-3'

SDC2 R: 5'-ACATGATTTATTCAGTAGTT-3'

TOX F: 5'-AGAAAGAGATTGAAAACAGC-3'

TOX R: 5'-CAAATTTACTTACAAACACC-3'

Fluorescence in situ hybridization (FISH)

The described primer sets were used for PCR screening of the bovine BAC library BBL 750 (Zhu et al., 1999). Standard PCR was performed using HotStarTaq DNA Polymerase (Qiagen) at an annealing temperature of 55 °C. The identified BACs were labeled with Biotin-16-dUTP and used for FISH on photographed QFH-banded cattle chromosomes. The hybridization followed the standard protocol (Pinkel et al., 1986). 200 ng probe DNA were annealed with 5 µg cattle C₀t-1 DNA and 20 µg salmon sperm DNA for 40 min at 37 °C in a pre-hybridization reaction to compete unspecific DNA sequences. The analysis of FITC fluorescence signals was done on propidium iodide stained chromosomes.

	SDC2	TOX
Probe names:	BBL_B750 B19268	BBL_B750 L03265
Probe type:	Bovine genomic DNA-containing BAC clone	
Insert size:	~ 100 kb	
Vector:	pBACe3.6 (BAC)	
Proof of authenticity:	PCR, DNA sequencing, NCBI-BLAST	
Gene reference:	BE217551	BE217422

Supported by grant GO-896/2-1 from the Deutsche Forschungsgemeinschaft and grant 000517-0293-1999 from the Texas Advanced Technology Program. The BAC library was constructed by Ross Miller and John L. Williams as a part of the EU framework IV BOREALIS project.

Received 22 January 2003; manuscript accepted 20 March 2003.

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Fig. 1 . Assignment of SDC2 and TOX by FISH on BTA14. **(left)** QFH-banded chromosomes prior to FISH; **(middle)** same chromosomes after FISH with gene specific bovine BAC clones; **(right)** Additional chromosome pairs with FITC signals.

Results

Mapping data

Most precise location:

Nucleotide position in human chromosome reference sequence:

Number of cells examined:

Number of cells with specific signal:

Location of background signals:

Mapping by FL

Number of chromosomes examined:

Mean location:

Bands encompassed:

Range:

Standard deviation:

SDC2

BTA14q22

NT_008046:

6605500 bp – 6605734 bp

12

1 (1), 2 (1), 3 (1), 4 (9) chromatids

per cell

none observed

TOX

BTA14q17

NT_008183:

5138581 bp – 5138369 bp

12

1 (0), 2 (2), 3 (2), 4 (8) chromatids

per cell

none observed

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