Assignment\(^1\) of murine placental cathepsin R to mouse chromosome bands 13B2–B3 by fluorescence in situ hybridization

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\(^1\) To our knowledge this is the first time this gene has been mapped by FISH.

Rationale and significance

Recent studies revealed several essential roles for papain-like cysteine proteases in human or animal pathophysiology by their activities in degrading unwanted proteins, generating bioactive peptides, or dissolving important extracellular matrix-proteins, which leads to the pathogenesis of several human diseases (Chapman et al., 1997; Dickinson, 2002; Motyckova et al., 2002; Yan et al., 2003; Liu et al., 2004). Cathepsin R (CTSR) is a lysosomal cysteine protease expressed primarily in mouse placenta (Sol-Church et al., 2000). Increased expression of cathepsin R correlated with the latter half of pregnancy (Ishida, et al., 2004), suggesting a role of this enzyme in nutrient or gas exchanges between maternal and fetal blood. However, a detailed characterization and its exact function in placenta remain to be investigated. We report here the isolation of a mouse cathepsin R genomic clone and chromosomal localization of the gene to chromosome 13B2–B3 with fluorescence in situ hybridization (FISH).

Materials and methods

Cloning of murine placental cathepsin R

To isolate mouse cathepsin R genomic clones, a bacterial artificial chromosome (BAC) genomic mouse library was screened by polymerase chain reactions using the sequence at both the 5'-end (sense primer: 5'-AAA TTT GTC GAT TGG CGA AAG AAA-3') and antisense primer 5'-ACA GTC CAC CAG GTT CTG CAC AGT-3') and 3'-end (sense primer: 5'-ACA AAC CAC TGT GGA ATT GCT TCA-3' and antisense primer: 5'-AGG TTG CTC TTC AGG GAC ACA AGT-3') of the murine cathepsin R cDNA (Sol-Church et al., 2000). This screening identified one BAC genomic clone of the murine cathepsin R. BAC genomic DNA was isolated using Qiagen Maxiprep kit (Qiagen) and directly utilized for DNA sequencing for further confirmation.

Probe name: mouse cathepsin R
Probe Type: mouse genomic DNA
Vector: Bacterial Artificial Chromosome
Insert Size: 40 kb
Gene symbol: Ctsr
Gene name: mouse cathepsin R
Proof of authenticity: DNA sequencing
Gene reference: GenBank accession number AY943668

Fluorescence in situ hybridization (FISH)

The whole murine cathepsin R genomic DNA was labeled with biotin or digoxigenin (Random Primer DNA Labeling Kit, Boehringer-Mannheim, Germany) and used for fluorescence in situ hybridization (FISH) of mouse metaphase chromosomes derived from spleen culture. Hybridization, washings, digital-image acquisition, processing and analysis, as well as the procedure for direct visualization of fluorescent signals to banded chromosomes were done as previously described (Zimonjic et al., 1995). Metaphases with specific hybridization signals were recorded and slides were rehybridized for spectral karyotyping (SKY, Applied Spectral Imaging, Karlshad, CA) analysis to unequivocally establish the identity of labeled chromosomes.
Results

Mapping data
Number of cells examined: 38
Number of cells with specific signal: 1(0), 2(2), 3(0), 4(28) chromatids per cell
Number of chromosomes examined: 58
Mean location: 13B2–B3

References