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Assignment[1] of Protease, Serine-Like 1 (PRSSL1) to human chromosome 19q13 by in situ hybridization and radiation hybrid mapping

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[1] To our knowledge this is the first time this gene has been mapped.

Rationale and significance

PRSSL1 cDNA encodes a 30.14 kilodalton secreted protein that shares 34–42% amino acid identity with several serine protease families (Liu et al., 1996). PRSSL1 expression was detected in normal and immortalized nontumorigenic mammary epithelial cells but was reduced or absent in numerous human breast cancer cell lines (Liu et al., 1996). Thus, PRSSL1 may play a role in suppression of tumorigenesis. To further investigate this, we have mapped and localized PRSSL1 to chromosome 19 band q13.3. Significantly, LOH studies on gliomas have shown a frequent allelic loss (53%) of chromosome 19q at marker D19S246 (Bicher et al., 1997), and a commonly deleted region (81%) at 19q13.2→q13.4 (Reifenberger et al., 1994). These data suggest the presence of a tumor suppressor gene.

Materials and methods

Based on the published PRSSL1 cDNA sequence (Liu et al., 1996), a PCR primer set (NES1F: 5'-TGCCCTCTCTGAACCTCAGTTTC-3' and NES1R: 5'-TCACCACCCACTGTGTTAAGCC-3'), located in the C-terminal region of the cDNA template, was used to identify genomic clones in an arrayed BAC library according to the manufacturer's protocols (Research Genetics). The screen yielded two BAC clones: RMC19B012 and RMC19B013. The presence of PRSSL1 sequence in the two isolated BAC clones was confirmed by Southern hybridization using a digoxigenin-labeled cDNA probe (data not shown).

The two PRSSL1 BAC clones were localized on human target chromosomes by fluorescence in situ hybridization (FISH) as described elsewhere (Stokke et al., 1995). Briefly, DNA was extracted from an overnight culture using a modified alkaline lysis method (Lee and Rasheed, 1990). The DNA was nick-translated with digoxigenin-dUTP (Boehringer Mannheim) using the Bio-Nick labeling system (BRL Life Technologies). Labeled probe was then hybridized to normal human metaphase chromosomes. Hybridized signal was detected with FITC-antidigoxigenin antibody (Boehringer Mannheim). The location of the hybridization signal on chromosome band 19q13 was determined by DAPI-banding and the FLpter fractional length measurements: 0.86 and 0.84 (RMC19B012 and RMC19B013, respectively) were determined by digital imaging microscopy (Sakamoto et al., 1995).

In order to refine the localization of PRSSL1, the GeneBridge 4 radiation hybrid panel was screened with a primer set, (NES1.1F: 5'-CGTGGTCACCAACAACATGATATG-3' and NES1.1R: 5'-GGCTTCTCTGGAATAAACCTTTGC-3') located within the last exon and 3'-UTR of the PRSSL1 cDNA template, according to the manufacturer's protocols (Research Genetics).

Probe names: RMC19B012 and RMC19B013
Probe type: genomic DNA
Insert size: Approximately 100 kb
Vector: pBeloBAC11
Proof of authenticity: Southern hybridization
Gene reference: Liu et al. (1996)

Results

Mapping data RMC19B012
Location: 19q13
Number of cells examined: 20
Number of cells with specific signal: 1 (0), 2 (0), 3 (0), 4 (20) chromatids per cell
Most precise assignment: 19q13.3
Location of background signals (sites with >2 signals): none observed
Chr. 19 average FLpter measurement (n = 13): Flpter ± S.E. = 0.86 ± 0.011

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Mapping data RMC19B013

Location: 19q13

Number of cells examined: 25

Number of cells with specific signal: 1 (0), 2 (0), 3 (1), 4 (24) chromatids per cell

Most precise assignment: 19q13.3

Location of background signals (sites with >2 signals): none observed

Chr. 19 average FLpter measurement (n = 7): FLpter ± S.E. = 0.84 ± 0.012

The results place PRSSL1 on the long arm of chromosome 19, 2.2 cR from the framework marker WI-5264 (Lod >3.0), telomeric to NIB1805 and centromeric to WI-5423 (Whitehead institute map). Based on GDB's comprehensive map, marker WI-5264 (D19S835) is estimated to be at 19q13.33. This location is in accordance with our Flpter measurements. On Lawrence Livermore National Laboratories recent cytogenetic map, we place PRSSL1 distal in band q13.3 centromeric to marker D19S246.

Southern hybridization and FISH results can be viewed, or the probes requested, at University of California, San Francisco Cancer Center/Lawrence Berkeley National Laboratory, Resource for Molecular Cytogenetics home page at http://www-rmc.lbl.gov.

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