Assignment¹ of porcine CD97 gene to the 1/2q21 → q22 region of the pig chromosome 2 with somatic cell hybrids

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

Rationale and significance

CD97 is a member of a novel subfamily of leukocyte proteins that are characterized by the presence of tandemly repeated extracellular epidermal growth factor (EGF)-like domains and a seven-span transmembrane region, known as EGF-TM7. To date, the EGF-TM7 group comprises CD97, EGF module-containing mucin-like hormone receptor (EMR) EMR1, EMR2, EMR3, and ETL in human, and CD97, F4/80 (EMR1) and EMR4 in the mouse (Kwakkenbos et al., 2002; McKnight et al., 1998; Stacey et al., 2002; Stacey et al., 2001). CD97 is induced rapidly on the surface of most leukocytes upon activation. So far, CD97 is the only EGF-TM7 family member of which a ligand has been identified. CD97 binds to its cellular ligand CD55 (decay accelerating factor), which protects several cell types from complement-mediated damage (Hannan et al., 1996). The functional consequences of CD97-CD55 binding are largely unknown, but previous data imply that CD97-CD55 interactions play a role in cellular activation, migration, and adhesion under inflammatory conditions (Vissler et al., 2002).

The CD97 structural gene is mapped to the 19p13.2 → p13.12 region on human chromosome 19. We have recently cloned the pig homologue of the CD97 antigen. Sequence comparison indicates that pig CD97 shares many structural features with human, cattle and mouse CD97 (Perez de la Lastra et al., 2003). To date, this is the first member of the EGF-TM7 family identified in the pig.

Materials and Methods

PCR reactions were performed using the following set of primers:

P1: 5'-CATCCTCTCCTCTCCCACTGTTCT-3'

P2: 5'-GCACCCGACCTCTTGTCTGTAG-3'

Primers P1 and P2 were designed to amplify a 317 bp sequence, located in the 3'-UTR region of the pig CD97 mRNA sequence (Gen Bank accession number AF413207).

The porcine somatic cell hybrid panel used consisted of 27 pig/rodent hybrids (including 19 pig/hamster and 8 pig/mouse hybrids) (Yerle et al., 1996; Robic et al., 1996). For genotyping of the hybrid panel, 25 ng of each cell line DNA and of each control DNA from the parental cells (hamster and mouse) were amplified with the pig CD97 primers. Cycling conditions were 95 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min for 35 cycles. The PCR products were resolved on a 2% agarose gel and individual cell lines were scored by the presence (+) or absence (–) of a fragment of the correct size. Statistical calculations of the assignment were performed using software previously developed (Chevalet et al., 1997).

Results

When the PCR reactions were carried out as described above, a porcine CD97-specific product was present in 3 hybrids (number 6, 21 and 25). No PCR amplification was found in the rest of the hybrids (Fig. 1) or in the DNA from the parental cell lines (hamster and mouse). This result allowed the localization of the pig CD97 gene to the region 1/2q21 → q22 of

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Fig. 1. Diagram representing the fragments of porcine chromosome 2 in each hybrid clone (Yerle et al., 1996; Robic et al., 1996). The chromosome fragments are shown as solid bars spanning the length of the fragment. The presence of fragments of pig chromosomes enables the definition of regions named by capital letters (A-F). Positive hybrids for CD97 were 6, 21 and 25, indicating that this gene is present in the region D or 1/2q21 → q22 of the chromosome.

the long arm of chromosome 2 with a probability of 0.6856, an error risk lower than 0.5 % and a maximal correlation of 1.0. No discordant clone was found for the regional localization (Fig. 1). These data demonstrate that the porcine CD97 gene is located on the region D or 1/2q21 → q22 of the long (q) arm of chromosome 2. Genomic mapping analysis has suggested a possible EGF-TM7 gene family on the human chromosome 19p13 region and on the corresponding syntenic regions of the mouse chromosomes 17 and 8 (Carver et al., 1999). In this region, members of the EGF-TM7 gene family map close to each other and are the probable products of a recent gene duplication event (Lin et al., 2000).

By comparative gene mapping, extended conservation of synteny between different mammalian species has become apparent. Recently, a high resolution comparative map was constructed for porcine chromosome 2. Comparison of the porcine RH map identified four conserved segments between SSC2 and HSA11, HSA19, and HSA5 (Rattink et al., 2001a). In addition, a porcine bacterial artificial chromosome (BAC) library was screened with 17 microsatellite markers and 18 genes previously assigned to pig chromosome 2 and several of these genes were located on human chromosome 11 and 19 (Rattink et al., 2001b). These findings add support to our localization data of CD97 gene in the pig.

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