Assignment¹ of the CD47 gene to pig chromosome band 13q42→1/2q46 with somatic cell hybridsY.E. Shahein,^a J.J. Garrido,^a M. Yerle,^b M.T. Roldan-Arjona,^c
J.M. Perez de la Lastra,^a and D.F. de Andres-Cara^a^aUnidad Mixta CSIC-UCO Marcadores Genéticos Moleculares, Facultad de Veterinaria, Córdoba (Spain);^bLaboratoire Génétique Cellulaire INRA, Castanet-Tolosan (France);^cDepartamento de Genética, Facultad de Ciencias, Córdoba (Spain)¹ To our knowledge this is the first time this gene has been mapped in pig.

Rationale and significance

CD47 is a integrin-associated protein originally discovered as a plasma membrane molecule that co-purified with the integrin $\alpha v \beta 3$ from leukocytes and placenta (Brown et al., 1990). CD47 is an unusual member of the immunoglobulin (Ig) superfamily of membrane proteins, with a single IgV-like domain at its N-terminus, a highly hydrophobic stretch with five membrane-spanning segments and an alternatively spliced cytoplasmic C-terminus ranging in length from 3 to 36 amino acids (Schwartz and Baron, 1999). In human, CD47 is a receptor for thrombospondin family members, a ligand for the transmembrane signaling protein SIRP α and a component of a supramolecular complex containing specific integrins, heterotrimeric G proteins and cholesterol (Brown and Frazier, 2001). Mouse, rat, porcine and bovine CD47 molecules have been cloned and show about 70% overall amino acid identity with the human molecule suggesting that this gene might play a similar role in different species.

Materials and methods

PCR reaction was performed using pig CD47-specific primers F 1745 (TGTCCTCCTGTTATTTGCTTCTGC) and R2335 (GTAGGTACCCCGCTGTCATCCATA) derived from the cDNA sequence of pig CD47 (GenBank reference: AF332698), which specifically amplify a 614-bp fragment located at the 3' nontranslated region of the gene. The porcine somatic cell hybrid panel consisted of 27 well characterized pig-rodent hybrids (including 19 pig-hamster and eight pig-mouse hybrids) (Yerle et al., 1996). For genotyping of the hybrid panel, 50 ng of DNA from each cell line and control sample (pig, hamster, mouse) were amplified using the CD47 primers. The PCR reaction was performed as previously described (Garrido et al., 1998). PCR products were evaluated on a 2% agarose gel and individual cell lines were evaluated for the presence or absence of a fragment of the correct size. Statistical calculations of the assignment were performed using software developed by Chevalet et al. (1997).

Results

Regional mapping results

Using the CD47 primers, we detected a 614-bp porcine-specific band in six of the 27 hybrids (hybrids 6, 7, 12, 16, 22 and 23) (Fig. 1). These results allowed regional assignment of CD47 to porcine chromosome 13 (SSC13) region q42→1/2q46 with a probability of 0.89, an error risk inferior to 0.5% and one discordant clone (hybrid 23). Human CD47 is located on the long arm of chromosome 3 (HSA3), band q13.1→q13.2 (Lindberg et al., 1994). Recently, Zoo-FISH and somatic cell hybrid panels have demonstrated extended synteny conservation between human chromosome 3 and pig chromosome 13 (van Poucke et al., 1999). This homology was confirmed with a com-

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parative map of HSA3 and SSC13 constructed using physically assigned pig sequence-tagged sites and also demonstrating extensive gene-order differences between man and pig within this large conserved syntenic group (Sun et al., 1999). These findings add support to our localization of CD47 on SSC13.

Fig. 1. Diagram representing the fragments of porcine chromosome 13 in each hybrid clone. The chromosome fragments are shown as solid bars spanning the length of the fragment. The presence of various chr 13 fragments enables the definition of regions named by capital letter. Positive hybrids for CD47 were 6, 7, 12, 16, 22 and 23, indicating that CD47 maps to region E (q42 → 1/2q46). Hybrid 23 was discordant.

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