Assignment¹ of the murine ankyrin-repeated protein gene (*Ankrd2*) to mouse chromosome 19C3→D1 and rat chromosome 1q51→q53 by fluorescence in situ hybridization

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

Rationale and significance

The human ANKRD2 gene was isolated from a cDNA library of human esophageal carcinoma cell line by immunoscreening (Moriyama et al., 2001), and independently cloned from two other laboratories in relation to skeletal muscle development and stretch-induced hypertrophy (Kemp et al., 2000; Pallavicini et al., 2001). It encodes an ankyrin-repeated protein highly homologous to the cardiac-restricted ankyrin repeat protein (CARP, now designated as ANKRD1), which is a nuclear protein and may serve as a transcriptional negative regulator for MLC-2v in cardiomyogenesis (Zou et al., 1997). Since ANKRD2 and ANKRD1 expression normally found in various muscle tissues were elevated in different types of muscle-related lesions (Pallavicini et al., 2001; Ishiguro et al., 2002; de Waard et al., 2003), these genes might have an important role not only in the normal myogenetic differentiation but also in the pathogenesis of muscle-associated disorders. We have previously mapped the human ANKRD2 on chromosome band 10q24 (Moriyama et al., 2001) and successively the mouse homolog of ANKRD2 was isolated (Tsukamoto et al., 2002). We report here the localization of *Ankrd2* on mouse and rat chromosomes by fluorescence in situ hybridization together with the determination of the gene order of *Ankrd2* and *Ankrd1* in human, mouse, and rat.

Materials and methods

DNA probe and chromosome preparation

A λ phage clone containing an approximately 15-kb insert of the mouse *Ankrd2* gene was isolated from a mouse genomic library (Stratagene) using the human ANKRD2 cDNA as a probe, and the clone was confirmed by direct DNA sequencing (Tsukamoto et al., 2002). As a reference probe, BAC clones of RP11-71F8, which contains the human ANKRD1, and RP23-123K14 and RP24-161O23 which contain the mouse *Ankrd1*, were purchased from Children's Hospital Oakland Research Institute (CHORI)-BACPAC Resources. The former was confirmed to contain the human ANKRD1 by PCR amplification of a STS marker RH98636 (UniSTS: 92563). The latter was also confirmed to contain the mouse sequence by PCR by using oligonucleotide primer sets of 5'-CAGCTCCTCTACTCTCAGTACCATC-3' (forward) and 5'-TACGTGGGATGACTCGCATTGCTGA-3' (reverse). The expected 345-bp product from the 5' region of *Ankrd1* cDNA was subcloned into the pGEM-T Easy Vector (Promega) and sequenced directly (data not shown).

Mouse and rat metaphase chromosomes were obtained from the embryonic stem cells of the 129 strain and from a rat fibroblast cell line, RAT1. Normal human metaphase spreads from peripheral blood mononuclear cells were prepared by standard procedures.

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed according to the method described previously (Satoh et al., 1993). Chromosomal assignments were confirmed by following hybridization experiments using FITC-labeled chromosome-specific painting probes (Cambio). Photo slides were scanned using EPSON ES-2000 (Tokyo, Japan), then FITC, rhodamine, and DAPI images were further processed using Adobe Photoshop Version 5.5 software.

Probe name: mouse *Ankrd2*

Probe type: mouse genomic DNA

Insert size: approximately 15 kb

Vector: Uni-ZAP XR

Proof of Authenticity: PCR, DNA sequencing

Gene reference: AB079548

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Results and discussion

Mapping data: mouse *Ankrd2*, rat *Ankrd2*

Most precise location: 19C3→D1, 1q51→q53

Nucleotide position in human chromosome reference sequence: 98997123 bp-99008224 bp, 98997123 bp-99008224 bp

Number of cells examined: 28, 11

Number of cells with special signal: 1(0), 2(8), 3(8), 4(12); 1(6), 2(5), 3(0), 4(0)

Location of background signals: none observed, none observed

We first determined the position of the mouse *Ankrd2* gene on mouse chromosome 19 at band C3→D1, confirming the predicted data on NCBI Entrez Map View (www.ncbi.nlm.nih.gov).

Fig. 1. Comparative chromosome mapping of the murine *Ankrd2* and *Ankrd1* genes in the mouse and the rat. Dual color FISH signals for *Ankrd2* (green) and *Ankrd1* (red) were seen for mouse chromosome 19 and rat chromosome 1 (left), along with the DAPI stained same panel (right). The ideograms show G-banded mouse chromosome 19 and rat chromosome 1 together with the possible locations for *Ankrd2* (vertical bar in green) and *Ankrd1* (vertical bar in red).

Fig. 2. Comparative physical map between ANKRD1 and ANKRD2 in human, mouse, and rat. Physical map showing the 6.7-Mb region between ANKRD1 and ANKRD2 in human (left) comparing with those corresponding segments in mouse (middle) and rat (right). Physical distances of each gene locus from the ANKRD1 gene are plotted with small horizontal bars and the numbers show the distances in Mb. Red and green arrows drawn on the left show the transcriptional direction of each gene. Orthologous gene loci are connected together by dotted lines, showing an extensive syntenic conservation in this region. The gene symbols are used as followed by the NCBI Entrez Map View. Gene symbols in parenthesis are still putative. The human ANKRD2 is located 2.3 Mb downstream of PDLIM1 and 6.7 Mb upstream of GSTTLp28 and the mouse *Ankrd2* is positioned between the two genes with similar gene intervals. The rat *Ankrd2* has not yet been precisely located on the physical map; however, it would localize between *Pdlim1* and *Gsto1* about 7 Mb upstream from *Alrp* (*Ankrd1*).

nih.gov/mapview). The same probe was applied for mapping of the rat counterpart, and a unique hybridization was detected on the distal region of the long arm of rat chromosome 1 that is syntenic to mouse chromosome 19. None of other rat chromosomes showed specific hybridization; therefore, we concluded the locus for rat *Ankrd2* to rat chromosome 1 at band q51 → q53.

Secondly, we attempted to map the murine homologs for ANKRD2 and ANKRD1 simultaneously on both mouse and rat chromosomes. The resulting gene order of the centromere-*Ankrd1*-*Ankrd2*-telomere direction observed in the mouse genome was inverted in the rat genome (Fig. 1). The hybridization signal for human ANKRD1 was detected on 10q23.2 → q23.3 and was always observed proximal to ANKRD2 signal; that is in good agreement with the updated physical map of NCBI Entrez Map View (accessed on Feb 25, 2004) but discrepant with its deduced cytogenetic map. Therefore, we propose a more precise localization for ANKRD2 from 10q23.31 → q23.32 to 10q24 in the updated data.

Finally, twelve human genes are located between ANKRD1 and ANKRD2 when comparing the gene alignment minutely (Fig. 2). In the restricted region, the sizes, the scattered distribution, and the transcriptional direction of syntenic genes are well conserved among the three species.

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