Original Articles

Chromosome Assignment of *Cd36* Transgenes in Two Rat SHR Lines by FISH and Linkage Mapping of Transgenic Insert in the SHR-TG19 Line

(SHR / *Cd36* / transgenic lines / FISH / linkage mapping)

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Abstract. The chromosome position of the *Cd36* insert was determined by FISH in two rat transgenic lines (SHR/Ola-TgN(EF1a*Cd36*)10Ipcv (SHR-TG10) and SHR/Ola-TgN(EF1a*Cd36*)19Ipcv (SHR-TG19). The *Cd36* transgene construct labelled with digoxigenin-11-dNTP was used as a probe in the FISH analysis. In accord with the previous finding that the SHR-TG10 harbours 6–8 copies of the transgene, the signals from both metaphase and interphase nuclei of SHR-TG10 preparations were rather strong and the probe hybridized to both copies of chromosome 1 at band q55. The probe hybridization to SHR-TG19 metaphase preparations also showed homozygosity of the transgene with localization of both copies to chromosome 11 at band q11. The signals were distinct but much weaker compared to the SHR-TG10, which again is in accord with the fact that the SHR-TG19 line harbours only a single copy of the transgene. In order to look for a possible impact of the insertion site neighbourhood upon the transgene phenotypic effect, we performed linkage mapping of the transgene in the SHR-TG19 line. By linkage mapping, the placement of the transgene to the proximal part of RNO11 was confirmed, the critical interval being 4 cM between *D11Rat20* and *D11Rat21*, in good agreement with the RH map. Within the close neighbourhood of the inserted *Cd36* transgene, there are several genes known to be expressed in kidney, and so the influence of some regulatory sequences enhancing kidney expression of the *Cd36* transgene can be envisaged.

Originally, the Cd36 was found on platelets as an integral membrane glycoprotein (Okumura and Jamieson, 1976) and underlies the human Nak[a] blood group polymorphism (Yamamoto et al., 1990). Cd36 functions as a thrombospondin and collagene receptor, binds oxidized low density lipoproteins (LDL), phospholipids and aged or *Plasmodium falciparum*-infected red blood cells. Cd36 was found to be expressed by several different cell types and involved in diverse physiological and pathological events, such as coagulation, host defence, inflammation, angiogenesis, lipid metabolism, scavenging, etc. (Telen, 2000; Febbraio et al., 2001). The implication of Cd36 in many different biological processes defines it as a multiligand scavenger receptor (Silverstein and Febbraio, 2000).

The *Cd36* gene is located to rat chromosome 4 at the peak of quantitative trait locus (QTL) linkage to spontaneously hypertensive rat (SHR) defects in glucose and fatty acid metabolism, triglyceridemia and hypertension, and it was recently shown to be defective in the spontaneously hypertensive rat strain SHR/OlaIpcv (Aitman et al., 1999). In order to analyse the causal involvement of *Cd36* in these disorders, we have derived several SHR transgenic lines, which express the introduced wild-type allele of the *Cd36* gene on the SHR background carrying the *Cd36* deletion variant (Pravenec et al., 2001). A relatively low level of wild *Cd36* expression in SHR-*Cd36* transgenic lines was shown to ameliorate metabolic disturbances compared to the intact SHR strain. In one of these lines, the SHR-TG19, with increased expression of wild-type *Cd36* in kidneys and liver, the blood pressure was significantly reduced (Pravenec et al., 2000). In order to elucidate the

Received March 19, 2002. Accepted April 11, 2002.

Grants from the Grant Agency of the Czech Republic 204/98/K015 and from the Swedish Medical Research Council, the Inga Britt and Anne Lundberg Research Foundation, and the Nilsson-Ehle Foundation are acknowledged. M. P. is an International Research Scholar of the Howard Hughes Medical Institute.

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Abbreviations: FISH – fluorescence *in situ* hybridization, QTL – quantitative trait locus, RH map – radiation hybrid map, SHR – spontaneously hypertensive rat.

position of transgenes in the genome of SHR recipients, the chromosomal position of the *Cd36* insert was determined by fluorescence *in situ* hybridization (FISH) in two transgenic lines (SHR-TG10 and SHR-TG19) and, in addition, linkage mapping of the *Cd36* insert in the SHR-TG19 line was performed using a segregating backcross population.

Material and Methods

Animals

The production and characteristics of SHR/Ola-TgN(EF1a*Cd36*)10Ipcv (SHR-TG10) and SHR/Ola-TgN(EF1a*Cd36*)19Ipcv (SHR-TG19) transgenic lines were described earlier (Pravenec et al., 2001). BN.*Lx.Cd36*.1K is a triple congenic strain (Šeda et al., 2002) that besides the differential segment of RNO8 (*Lx*) of PD/Cub origin and RNO20 (RT1) of SHR/OlaIpcv origin carries a short SHR chromosomal segment of RNO4 with the deletion variant of *Cd36* (Šeda et al., 2002).

The backcross population was prepared by mating F_1(SHR-TG19 x BN.*Lx.Cd36*.1K) x BN. Lx.*Cd36*.1K. SHR-TG19 animals were homozygous transgene carriers and so F_1 hybrids were hemizygous.

FISH

FISH was performed essentially according to Pinkel et al. (1986) with minor modifications (Helou et al., 1998). In brief, the transgenic construct clone (1 μg) was labelled with digoxigenin-11-dUTP (Boehringer Mannheim, Germany) by nick translation (Life Technologies Inc., Gaithersburg, MD). The appropriate length (100 to 500 bp) of the nick-translated fragments was monitored by checking the nick translation products on a 1% agarose gel. Eight hundred ng of labelled DNA were mixed with 50 μg unlabelled rat c_0t1 DNA, which was included in order to suppress probe hybridization to repetitive sequences. The probe DNA mixture was ethanol precipitated and dissolved in hybridization buffer (50% formamide, 2 x SSC, 10% dextran sulphate). After denaturation, the probe mixture was applied to rat C-metaphase preparations from bone marrow, which were prepared as described (Sladká et al., 1992). Rat metaphase chromosome slides were previously denatured at 73°C for 2 min in 70% formamide, 2 x SSC. Hybridization was allowed to proceed in a moist chamber for 48 h at 37°C. Subsequently, the slides were washed for 15 min at 45°C in 55% formamide, 2 x SSC, and then in 2 x SSC (pH 7.0) at 43°C for 9 min. The labelled probe molecules were detected with FITC antidigoxigenin (Oncor, Inc., Gaithersburg, MD) and the slides were washed 3 times in PBS and 0.1% Nonidet NP-40. Finally, chromosome spreads were counterstained with 4,6-diamidino-2-phenylindole (DAPI; 0.5 μg/ml) in an antifade solution (Vectashield®, Vector Laboratories, Inc., Burlingame, CA). The images were captured using the Leica DM RXA in combination with the Q-FISH software for microphotography.

Cd36 genotyping

Primers for PCR were designed in putative exons 5 and 7, respectively (forward RCD36FAT355F: 5'-AGT TCG CTA TTT AGC CAA GG-3' and reverse RCD36FAT675R: 5'-AGG ATA AAA CAC ACC AAC TGT-3'), to amplify a fragment of 328 bp from the intronless transgenic construct. Hemizygous transgene carriers are positive and non-carriers are negative. Amplification of an about 800 bp fragment from the *Cd36* gene on chromosome 4 served as an internal control (Fig. 1).

Linkage mapping

Seventy-five backcross BN.*Lx.Cd36*.1K x F_1(BN.Lx.*Cd36*.1K x SHR-TG19) animals were genotyped. Triple congenic strain BN.*Lx.Cd36*.1K was used for the derivation of the backcross progeny because multiple microsatellite polymorphisms with the SHR strain could be exploited for genotyping. Genomic DNA was isolated from tail biopsy by phenol-chloroform extraction and ethanol precipitation. Primers for rat microsatellite markers (*D11Mit1, D11Mit4, D11Rat20, D11Rat21, D11Rat28, D11Rat29, D11Rat40* and *D11Rat79*) were obtained from Research Genetics or synthesized by Gibco BRL according to published sequences (Rat Genome Database – http://rgd.mcw.edu/). PCR and PAGE analysis were performed according to Pravenec et al. (1996). Map Manager QTX version 12 (http://mcbio.med.buffalo.edu/mapmgr.html) was used for linkage evaluation.

Results and Discussion

1/ The assignment of Cd36 insert in the SHR-TG10 and SHR-TG19 transgenic lines by FISH

FISH was used to determine the chromosomal location of *Cd36* inserts in the SHR-TG10 and SHR-TG19 transgenic lines. The transgene construct was used as a probe in the FISH analysis. In the SHR-TG10 line, the probe hybridized to both copies of chromosome 1 at band q55 (Fig. 2a). The signals from both metaphase and interphase nuclei were rather strong, which was in good accord with our previous finding that the SHR-TG10 line harbours 6–8 copies of the transgene (Pravenec et al., 2001). The probe hybridization to the SHR-TG19 metaphase preparations showed homozygosity of the transgene and localization to both copies of chromosome 11 at band q11 (Fig. 2b). The signals were distinct but much weaker than in the SHR-TG10, which is in agreement with the fact that the SHR-TG19