Assignment¹ of the giant panda MHC class II gene cluster to chromosome 9q by fluorescence in situ hybridization

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Manuscript received 20 September 2004; accepted for publication by M. Schmid 25 November 2004.

¹ To our knowledge this is the first time this gene cluster has been mapped in the giant panda.

Rationale and significance

The major histocompatibility complex (MHC) is a highly polymorphic gene superfamily and is divided into class I, class II and class III regions each of which forms a gene cluster in the same chromosome (Gunther and Walter, 2001). The MHC extreme polymorphism is driven and maintained by a long-standing battle for supremacy between the immune system and infectious pathogens (the MHC sequencing consortium, 1999).

Despite the importance of resisting pathogen infections, the MHC of the giant panda (*Ailuropoda melanoleuca*) has received little attention so far. Considering that the high mortality (22%) in captive pandas results from infectious diseases (Qiu et al., 1993), it is vital to conduct MHC studies and incorporate them into the panda breeding plans. Here, the assignment of the MHC class II gene cluster was carried out for the giant panda.

Materials and methods

Chromosome preparation

Fibroblast cells of the giant panda were kindly provided by the State Key Laboratory of Reproduction Biology (SKLRB) of the Institute of Zoology, Chinese Academy of Science, Beijing, P.R. China. Chromosome preparations were made following standard procedures (Nie et al., 2002). Chromosome nomenclature and numbering were performed according to previous protocols (Wurster-Hill and Bush, 1980; Nash and O'Brien, 1987; Nash et al., 1998).

Clone screening

The MHC class II gene cluster comprises DP, DQ and DR gene families (Gunther and Walter, 2001). A primer pair (forward: 5'-CGA CTC AGA TGA CCA CAT TG-3'; reverse: 5'-GGG GAC ACA TAC TGT TGG TAG-3') was modified from bovine DQA locus of the class II gene cluster (Hess et al., 1999) and used to screen a bacterial artificial chromosome (BAC) library of the giant panda. We identified three positive clones 59G10, 71F2 and 77F8 and verified that the three recombinant BAC clones contained DQ genes of the MHC class II gene cluster using shotgun sequencing. The sizes of the positive clones were determined by pulsed-field gel electrophoresis using the CHEF apparatus (Bio-Rad).

Fluorescence in situ hybridization

The DNA of clone 59G10 was extracted using a large-construct kit (Qia-gen) and was labeled with digoxigenin-11-dUTP (Roche) by nick-translation (Langer et al., 1981). FISH procedures were carried out as described by Helou et al. (1998) and the labeled probes were detected by anti-digoxigenin-rhodamine antibody (Roche). Finally, the giant panda metaphase chromosomes were counterstained with 4,6-diamidino-2-phenylindole (DAPI) in an anti-fade solution (Vector laboratories). Slides were examined under an Olympus BX61 fluorescence microscope. Chromosome and FISH signal images were captured using a DVC camera. The DAPI images were pseudo-colored and merged using registration software IPLab v3.61 (Scientific image processing Scanalytics Inc.). Final image adjustments were done with Adobe Photoshop 6.01 software.

Probe name: 59G10

Probe type: Bacterial Artificial Chromosome (BAC)

Insert size: approximately 150 kb

Vector: pBeloBAC11

Proof of authenticity: sequencing

This work was supported by a grant from the State Key Basic Research and Development Plan of P.R. China (G2000046906) and a special grant from the State Forestry Administration of P.R. China. We thank Professor Da-Yuan Chen and Ms. Zhao-Hui Kou for their help in culturing the fibroblast cells. The FISH experiments were performed at the Institute of Genetics and Developmental Biology, Chinese Academy of Science. We would also like to thank Professor Zhu-Kuan Chen and Dr. Wen-Li Zhang for their guidance and kind help in performing FISH.

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Results

Mapping data:

Human chromosome reference sequence: 6p21.3

Location: 9q (Fig. 1)

Number of cells examined: 15

Most precise mapping: centromeric 9q

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Fig. 1. FISH mapping of a DNA probe prepared from the 59G10 clone containing partial sequences of the MHC class II gene cluster. **(a)** FISH signal image; **(b)** The same metaphase chromosomes with the hybridization signals on inverted DAPI-banded chromosomes. Arrows indicate chromosomes with hybridization sites.